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MEDICINSKE ZNANOSTI

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Sadržaj

K. Kovačić, Š. Spaventi:
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Značenje endokrinoloških pretraga u ranom karcinomu dojke
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Oral health conditions and treatment needs for youth from different regions of Yugoslavia
Stanje zdravlja usne šupljine i potreba liječenja omladine u raznim područjima Jugoslavije

P. Pavlović, F. Dobrila, S. Georgijev, I. Pavlović-Ružić i F. Kučan:
Principi liječenja ranog raka dojke u kliničkom bolničkom centru Rijeka
The principles of treatment of early breast cancer in the Clinical Medical Center Rijeka
Significance of serum concentrations of CA 15^{-3}, TPA, CEA and Ferritin in patients with breast cancer
Značenje koncentracije u serumu CA 15^{-3}, TPA, CEA i Firritina u bolesnica sa rakom dojke

D. Ikić, I. Padovan, N. Pipić, M. Knežević, B. Rode, B. Nekić, V. Petrovečki and N. Đaković:
Subtumoral and intratumoral application of human natural leucocytic interferon and recombinant HuIFN alpha 2c
Subtumorska i intratumorska aplikacija ljudskog naravnog leukocitarnog interferona i rekombinata HuIFN alpha 2c kod bolesnika sa rakom glave i vrata

D. Ikić, I. Padovan, M. Knežević, N. Pipić and N. Đaković:
Application of interferon in patients with carcinoma baseocellulare
Aplikacija interferona kod bolesnika sa bazeocelularnim karcinomom

D. Ikić, I. Padovan, M. Knežević, N. Pipić and N. Đaković:
Application of interferon in patients with carcinoma planocellulare
Aplikacija interferona kod bolesnika sa planocelularnim karcinomom

D. Ikić, Š. Spaventi, M. Knežević, B. Pokrajac and N. Đaković:
Melanomas. Therapy with recombinant interferon HuIFN alpha 2c. Preliminary report
Melanomi. Terapija sa rekombinantnim interferonom HuIFN alpha 2c. Prethodno saopćenje

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SADRŽAJ

TABLE OF CONTENTS

K. Kovačić, Š. Spaventi: Uloga scintigrafije kosti u ranom otkrivanju metastaza karcinoma dojke 7

K. Kovačić and Š. Spaventi: The role of bone scintigraphy in the early detection of breast cancer 13

V. Stančić, B. Vizner i M. Sekso: Značenje endokrinoloških pretraga u ranom karcinomu dojke 15

V. Stančić, B. Vizner and M. Sekso: The role of hormonal assessments in early breast cancers 25

J. Aurer-Koželj, K. Srdjak-Jorgić and M. Vouk: Oral health conditions and treatment needs for youth from different regions of Yugoslavia . . 27

J. Aurer-Koželj, K. Srdjak-Jorgić i M. Vouk: Stanje zdravlja usne šupljine i potreba liječenja omladine u raznim područjima Jugoslavije . . . 38

P. Pavlović, F. Dobrila, S. Georgijev, I. Pavlović-Ružić i F. Kučan: Principi liječenja ranog raka dojke u kliničkom bolničkom centru Rijeka . . 41

P. Pavlović, F. Dobrila, S. Georgijev, I. Pavlović-Ružić and F. Kučan: The principles of treatment of early breast cancer in the Clinical Medical Center Rijeka 45

D. Vrbanec, Lj. Lukinac, B. Pokrajac and Š. Spaventi: Significance of serum concentrations of CA 15^{-3}, TPA, CEA and Ferritin in patients with breast cancer 47

D. Vrbanec, Lj. Lukinac, B. Pokrajac i Š. Spaventi: Značenje koncentracije u serumu CA 15^{-3}, TPA, CEA i Firritina u bolesnica sa rakom dojke . 52

D. Ikić, I. Padovan, N. Pipić, M.Knežević, B. Rode, B. Nekić, V. Petrovečki and N. Đaković: Subtumoral and intratumoral application of human natural leucocytic interferon and recombinant HuIFN alpha 2c . . . 55

D. Ikić, I. Padovan, N. Pipić, M. Knežević, B. Rode, B. Nekić, V. Petrovački i N. Đaković: Subtumorska i intratumorska aplikacija ljudskog naravnog leukocitarnog interferona i rekombinata HuIFN alpha 2c kod bolesnika sa rakom glave i vrata 70

D. Ikić, I. Padovan, M. Knežević, N. Pipić and N. Đaković: Application of interferon in patients with carcinoma baseocellulare 73

D. Ikić, I. Padovan, M. Knežević, N. Pipić i N. Đaković: Aplikacija interferona kod bolesnika sa bazeocelularnim karcinomom 84

D. Ikić, I. Padovan, M. Knežević, N. Pipić and N. Đaković: Application of interferon in patients with carcinoma planocellulare 87

D. Ikić, I. Padovan, M. Knežević, N. Pipić i N. Đaković: Aplikacija interferona kod bolesnika sa planocelularnim karcinomom 92

D. Ikić, Š. Spaventi, M. Knežević, B. Pokrajac and N. Đaković: Melanomas. Therapy with recombinant interferon HuIFN alpha 2c. Preliminary report 99

D. Ikić, Š. Spaventi, M. Knežević, B. Pokrajac i N. Đaković: Melanomi. Terapija sa rekombinantnim interferonom HuIFN alpha 2c. Prethodno saopćenje 103

KOVAČIĆ KSENIJA I ŠIME SPAVENTI

ULOGA SCINTIGRAFIJE KOSTI U RANOM OTKRIVANJU METASTAZA KARCINOMA DOJKE

UVOD

Osnovni preduvjet za uspješno liječenje onkološkog bolesnika je rano postavljanje dijagnoze, kao i pravilna procjena proširenosti bolesti. Zadnjih petnaestak godina (od kada su u upotrebi fosfatni spojevi obilježeni tehnecijem) u sklopu dijagnostičkog postupka, kao i kasnije u praćenju toka bolesti, odnosno kontroli efekta terapije, vrlo važnu ulogu ima i scintigrafija kosti za koju se s pravom može reći da je rutinska pretraga u kontroli onkološkog bolesnika. Tome pridonosi njena vrlo visoka osjetljivost, a što znači da se patološke promjene u kostima na scintigramu vide znatno ranije negoli na rendgenogramu.

METODA

Bolesnicima je nakon prethodne blokade štitnjače Irenatom, i. v. injiciranо 740 MBq (20 mCi) 99 mTC—MDP (methilendiphosphonat) domaće proizvodnje. Sniman je cijeli skelet gama kamerom 3 do 4 sata nakon injiciranja.

PRIKAZ POJEDINIH SLUČAJEVA

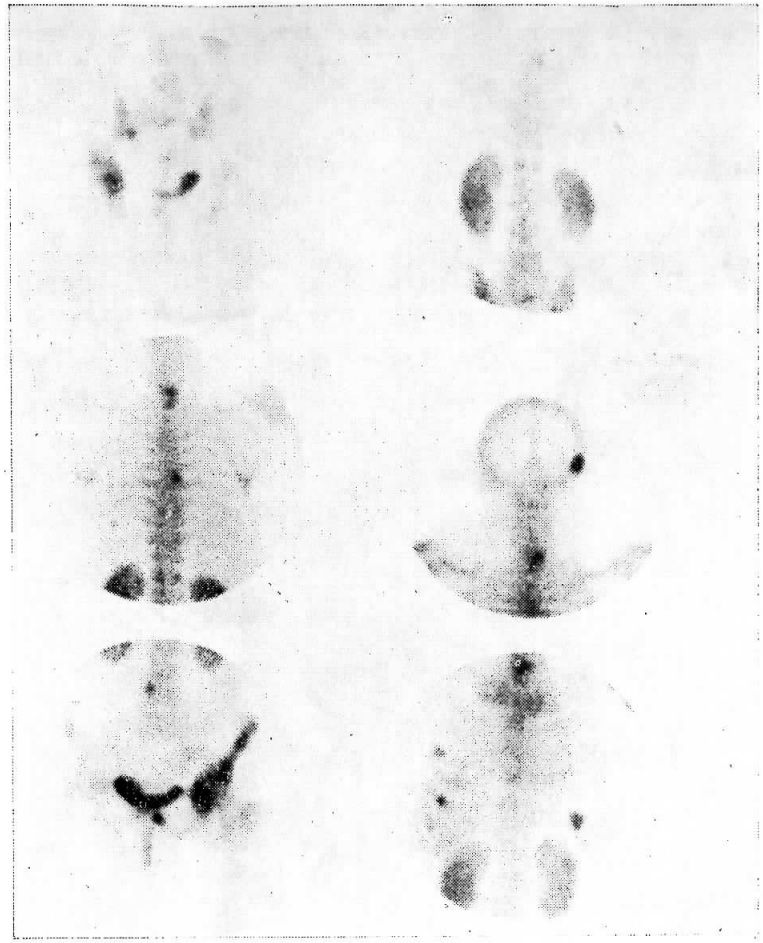
1. *Bolesnica F. A.* — 59 godina; zbog malignog procesa amputirana desna dojka 5 godina ranije. Bolovi u torakalnoj kralježnici unatrag nekoliko mjeseci. Uredan rtg nalaz. Učinjen scintigram pokazuje multipla mjesta patološkog nakupljanja kako u torakalnoj kralježnici, tako i u ostalim dijelovima skeleta, a što upućuje na multiple metastaze u kostima. (Sl. 1).

2. *Bolesnica G. B.* — 46 godina; amputirana lijeva dojka dvije godine ranije. Unatrag 4 mjeseca vrlo jaki bolovi u lijevom kuku. Obzirom na uredan rtg nalaz upućena na fizikalnu terapiju, nakon koje su bolovi još intenzivniji. Ponovljen rtg i nadalje uredan. Učinjen scintigram koji osim u području lijevog kuka pokazuje patološko nakupljanje RI i u ostalim dijelovima skeleta, a što upućuje na multiple metastaze u kostima. (Sl. 2).

Slika 1. Bolesnica F. A., 59 g. Multipla mjesta patološkog nakupljanja radioindikatora — torakalna i lumbalna kralježnica. Rtg nalaz uredan. Bolovi u torakalnoj kralježnici.

3. *Bolesnica H-B. M.* — 37 godina; dojka amputirana dvije godine ranije. Upućena je na scintigrafiju kosti zbog intenzivnih bolova u desnom kuku, a urednog rtg nalaza. Unatoč patološkom nalazu na scintigramu, ne samo u području desnog kuka, već i u ostalim dijelovima skeleta, a što upućuje na multiple metastaze u kostima, upućena je na fizikalnu terapiju. Scintigram ponovljen 4 mjeseca kasnije pokazuje izrazitu progresiju, a bolesnica se osim na bolove u desnom kuku sada žali i na bolove u gotovo svim kostima. Rtg nalaz i nadalje negativan. (Sl. 3).

Koliko je važno za pravilnu procjenu nalaza imati bazični scintigram, pokazuje slučaj broj 4. Radi se o bolesnici S. Z. — 70 godina, koja je prvi scintigram skeleta učinila 4 godine ranije, a zbog amputacije lijeve dojke 7 godina prije prvog scintigrama. Obzirom na vrlo inhomogeni prikaz kralježnice

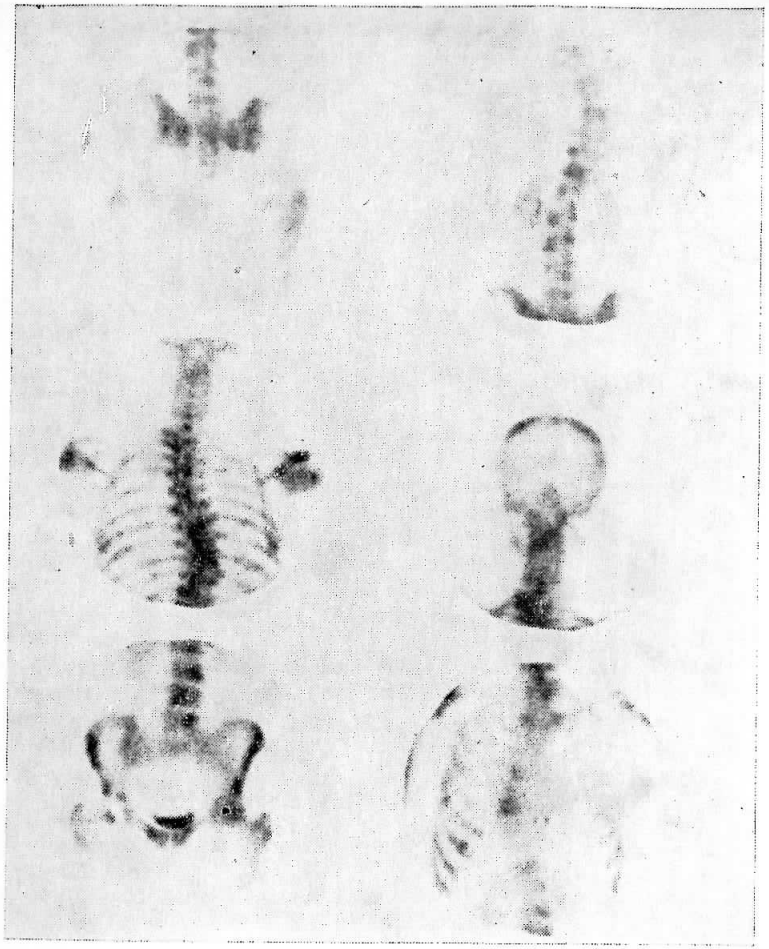


Slika 2. Bolesnica G. B., 46 g. Multiple metastaze u kostima. Rtg nalaz uredan. Bolovi u lijevom kuku.

preporučena je ciljana rtg obrada jer se moglo raditi i o jako izraženim degenerativnim promjenama. Bolesnicu gubimo iz vida za slijedeće 4 godine, a onda ponovo dolazi, sada radi malignog procesa i u drugoj dojci, te preoperativnog scintigrama. Kako je sada učinjen scintigram bez ikakvih promjena u odnosu na prethodni, a rtg nalaz prikazuje jako izražene degenerativne promjene, moglo se sa velikom vjerojatnošću kazati da nema scintigrafskih znakova koji bi upućivali na metastaze u kostima. (Sl. 4).

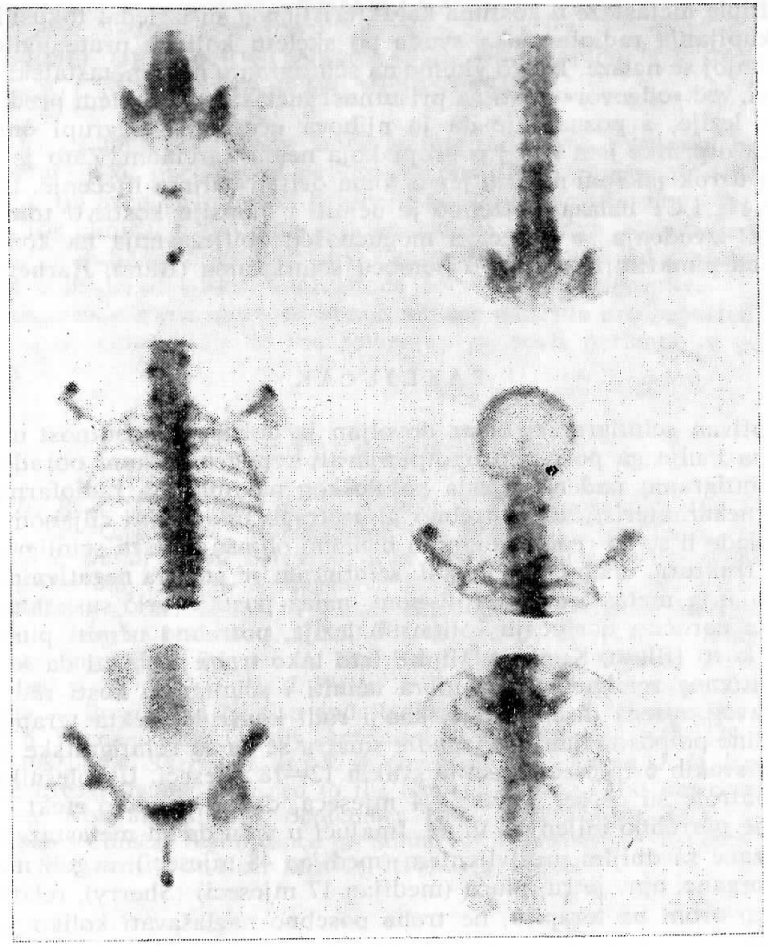
DISKUSIJA

Na prisutnost metastaza koštano tkivo reagira obično stvaranjem novog, ili tzv. »reaktivnog« koštanog tkiva. Procesi razaranja i stvaranja kosti teku



Slika 3. Bolesnica H. B. M., 37 g. Multiple metastaze u kostima. Uredan rtg nalaz. Bolovi samo u desnom kuku.

paralelno u različitim omjerima. Lezije u ranom stadiju bolesti izazivaju tako male promjene da se ne mogu vidjeti na rendgenogramu. Međutim, nakupljanje radiofarmaka na takvom mjestu je 2—3 puta veće od normale, a što u praksi znači da su lezije na scintigramu vidljive, 3, 6, pa i 18 mjeseci ranije negoli na rendgenogramu. Zato je scintigram pozitivan ne samo prije rendgenograma, nego i prije kliničkih simptoma, kao i prije promjena u laboratorijskim nalazima (Blum). Zato u bolesnica sa karcinomom dojke nije neuobičajen nalaz pozitivnog scintigrama, a negativnog rendgenograma, dok je obratno izuzetno rijetko. Isto je tako nađeno da samo oko 65% bolesnica sa metastazama karcinoma dojke u kostima ima bolove (Blum, Kagan). Iz svega proizlazi da uredni rtg, laboratorijski i klinički nalazi u bolesnica sa karcinomom dojke ne smije ni u kojem slučaju biti kontraindikacija za scintigrafiju kosti.



Slika 4. Bolesnica S. Z., 70 g. Inhomogeni prikaz kralježnice i lijevog kuka. Scintigram u razmaku od 4 godine. Nepromijenjen rtg. Izražene degenerativne promjene bez scintigrafskih znakova metastaza u kostima.

Postoje i u svijetu dileme da li pretragu raditi u svih bolesnika, znači i u onih u kojih je bolest otkrivena u ranom stadiju, ili samo u onih sa uznapredovalom bolesti (Blum, Bunkler). Mi smo prihvatili stav onih prvih koji smatraju da je u svih bolesnika scintigrafija kosti rutinska pretraga, a bez obzira na stadij bolesti, imajući u vidu nespecifičnost same metode. Naime, vrlo je važno imati bazični scintigram koji je moguće uspoređivati sa daljnim kontrolnim i to zato što i benigne bolesti kosti i zglobova mogu nekada simulirati metastaze tako da je pravilna interpretacija moguća jedino praćenjem takvih bolesnika. Što se tiče specifičnosti, ona se povećava kada je u pitanju točno određena grupa bolesnika, kao što su to u našem slučaju bolesnici sa karcinomom dojke i kada postoji dobra suradnja sa kliničarima.

Multiple metastaze u kostima karakterističnog su izgleda: fokusi intenzivnog nakupljanja radiofarmaka svuda po skeletu koji ne prate uvijek oblik kosti u kojoj se nalaze. To što vidimo na scintigramu nisu metastatski depoziti kao takvi, već »odgovor« kosti na prisutnost metastaza. Problem predstavljaju solitarne lezije, a poznato je da je njihova učestalost u grupi onkoloških bolesnika otprilike ista kao i u grupi koja nema karcinom. Zato je vrlo važno naći uzrok takvom nalazu, jer o tome ovisi i daljnje liječenje. U slučaju urednog rtg i CT nalaza, potrebno je učiniti i biopsiju kosti. U tom slučaju preciznost izvođenja se povećava mogućnošću obilježavanja na koži mjesta koje treba punktirati, a upravo pomoću scintigrama (Blum, Harbert, Mink, Tumeh).

ZAKLJUČAK

Negativan scintigrafski nalaz dovoljan je dokaz za odsutnost metastaza u kostima i nije ga potrebno upotpunjavati rendgenološkom obradom. Ako su na scintigramu nađena mjesta patološkog nakupljanja radiofarmaka, ali je nalaz nekarakterističan, potrebno je pretragu upotpuniti ciljanom rtg obradom. Nađe li se na rendgenogramu benigno objašnjenje za scintigrafski nalaz, npr. fraktura, upala, infarkt i sl. scintigram se smatra negativnim u smislu postojanja metastaza. U protivnom, nalaz postaje vrlo suspektan pa je nekada, a naročito u slučaju solitarnih lezija, potrebno učiniti punkciju ili biopsiju kosti (Blum, Kunkler, Mink). Isto tako treba naglasiti da se i u slučaju pozitivnog rendgenograma mora učiniti i scintigram kosti radi utvrđivanja pravog opsega diseminacije, kao i radi kontrole efekta terapije. Prve dvije godine po postavljanju dijagnoze smatra se da su scintigrafske kontrole potrebne svakih 6 mjeseci, kasnije svakih 12—18 mjeseci. U bolesnika na terapiji kontrole su češće, svaka 3—4 mjeseca, da bi se vidio efekt terapije, da li ju je potrebno mijenjati ili ne. Imajući u vidu da su metastaze u kostima povezane sa duljim preživljenjem (medijan 48 mjeseci), negoli metastaze u druge organe, npr. jetru, pluća (medijan 17 mjeseci) (Sherry), relativno dobrim odgovorom na terapiju, ne treba posebno naglašavati koliko je važno rano postaviti dijagnozu i rano započeti sa terapijom. Mogućnost ranog postavljanja dijagnoze postoji, pa je velika greška i neodgovornost ne iskoristiti je, već se oslanjati samo na rendgenske i laboratorijske kontrole, a njihov uredan nalaz smatrati dovoljnim dokazom za odsutnost metastaza u kostima.

SAŽETAK

Scintigrafija kosti je zbog svoje visoke osjetljivosti već cijeli niz godina rutinska pretraga u kontroli onkološkog bolesnika. Pozitivan scintigrafski nalaz prethodi rengenološkom 3, 6, pa i 18 mjeseci, također i kliničkim simptomima, kao i laboratorijskim nalazima. Zato uredni rendgenološki, laboratorijski i klinički nalazi u bolesnica sa karcinomom dojke ne smiju ni u kojem slučaju biti kontraindikacija za scintigrafiju kosti.

U članku je prikazano nekoliko slučajeva bolesnica sa karcinomom dojke, te je dan i općenit prikaz o ulozi scintigrafije kosti u praćenju takvih bolesnika.

SUMMARY

THE ROLE OF BONE SCINTIGRAPHY DETECTION OF BREAST CANCER METASTASIS

Due to its high sensitivity, bone scintigraphy has routinely been used in the follow-up of oncological patients. A positive scintigraphic finding may appear 3, 6 or even 18 months before a positive x-ray finding; the same is also true for clinical symptoms and laboratory findings. Normal x-ray, laboratory and clinical findings in breast cancer patients should therefore by no means be considered counterindications for bone scintigraphy.

In this article some cases of breast cancer patients are reported and the role of bone scintigraphy in the follow-up of such patients in general is discussed.

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VLADIMIR STANČIĆ, BRANKA VIZNER, I MLADEN SEKSO

ZNAČENJE ENDOKRINOLOŠKIH PRETRAGA U RANOM KARCINOMU DOJKE

UVOD

Dojka je osnovno biološko obilježje sisavaca i omogućuje im kao vrsti, autonomiju o okolini i visoku vjerojatnost othranjivanja mladunčadi, a time i sigurnije preživljenje vrste. Dojenjem je osiguran optimalan razvoj i jedinke i evolucija vrste. Zbog niza socijalnih, ekonomskih, nutritivnih i bioloških razloga karcinom dojke je danas najčešći solidni tumor u žena i najčešći uzrok smrti od raka u ženskoj populaciji.

Kako su tumorske stanice potomci zdravih stanica, one često zadržavaju i neke biokemijske karakteristike roditelja.

Pojedine stanice karcinoma dojke mogu sačuvati hormonsku ovisnost o steroidnim i nesteroidnim regulatorima proliferacije pa se zato karcinom dojke ubraja u klasične hormon-ovisne tumore. Endokrinološke manipulacije mogu biti korisne i u ranom (lokaliziranom) kao i kasnom (sistemnom) karcinomu dojke.

Osnova endokrinološkim manipulacijama bila je niz godina empirijska. Današnja saznanja o učinku hormona na zdrave i tumorske stanice dojke, kao i spoznaje o međuodnosu hormona i protuonkogena te receptora za faktore rasta, daju nade u još racionalniji pristup liječenju karcinoma dojke.

MEHANIZMI NASTANKA HORMON OVISNIH TUMORA

Hormon-ovisni tumori obuhvaćaju oko 30% bolesnika s rakom (1), a učestalost pojedinih lokalizacija ovih tumora prikazana je na tablici 1.

Pokusi na životinjama, endokrinološka ispitivanja kao i epidemiološki podaci nedvojbeno govore da hormoni i čimbenici rasta mogu sami ili udruženi s kancerogenim tvarima potaknuti tumorsku transformaciju i proliferaciju stanica u ciljnim organima. U pojednostavljenoj shemi o međusobnim odnosima hormona hipofize i njihovih glavnih sekundarnih i tercijarnih ciljnih organa prikazani su klasični putevi potencijalne indukcije i tumora u hormon-ovisnim organima (Tablica 2).

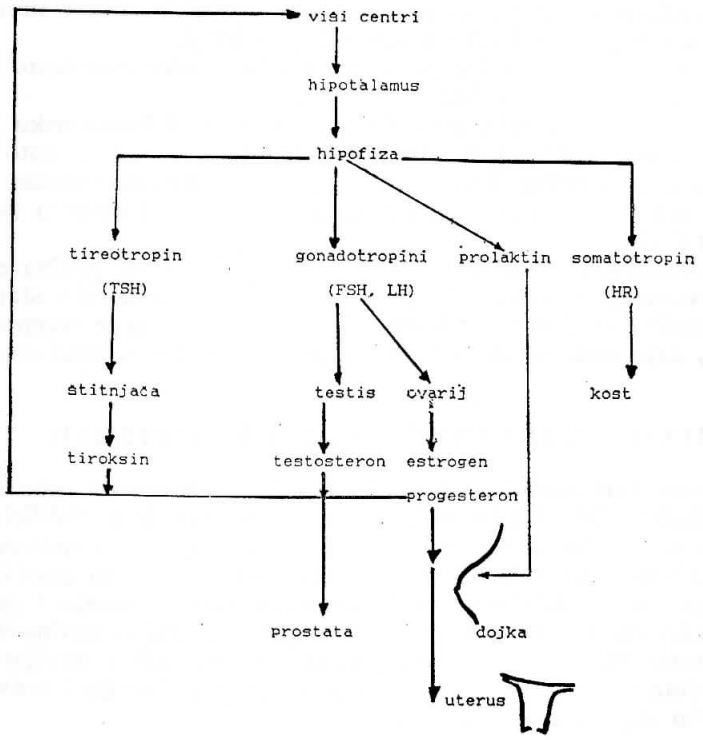
Tablica 1

GODIŠNJA INCIDENCIJA HORMON-OVISNIH NEOPLAZMI (PRILAGOĐENA DOBI). PREGLED III NACIONALNOG IZVJEŠTAJA O RAKU U SAD 1969. — 1971.

Lokalizacija	*muškarci*	*žene*
dojka	0,7	73,8
endometrij		22,2
ovarij		13,9
testis	3,1	
prostata	60,6	
štitnjača	2,1	5,0
kost	1,0	0,7
Ukupno	67,5	113,6
Ostali tumori	346,7	270,2

Tablica 2

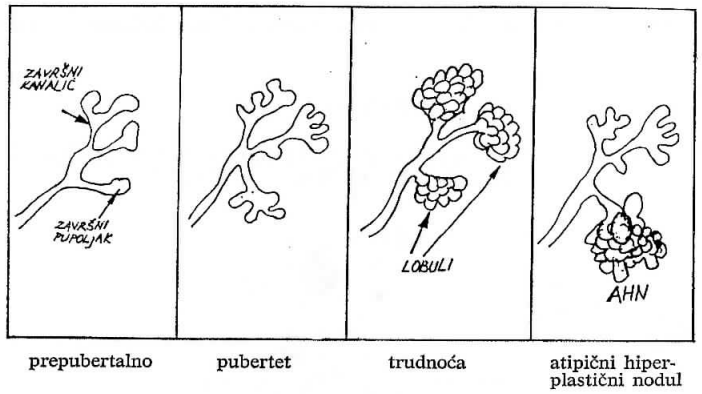
HORMONI HIPOFIZE I NJIHOVE GLAVNE CILJNE ŽLIJEZDE



Osjetljivost pojedinih stanica u ciljnim organima prema promotornim kancerogenim učincima hormona ovisna je o lokalizaciji stanica unutar ciljnog organa. To potvrđuje najveća učestalost karcinoma dojke (u žena i eksperimentalnih životinja) u terminalnim i/ili intralobularnim duktulima. Ovi terminalni kanalići tijekom trudnoće i dojenja diferenciraju se u sekretorne lobule. Spomenuta diferencijacija i transformacija obuhvaća takozvane matične stanice dojke koje su najosjetljivije na kancerogene poticaje. Rizik nastanka karcinoma raste proporcionalno broju cikličkih estrogenih stimulusa. To objašnjava smanjeni rizik nastanka karcinoma dojke u žena s ranom prvom trudnoćom i laktacijom jer su se u njih vrlo rano do kraja diferencirale matične stanice (1, 2). Histološke studije preneoplastičnih tvorbi i ranog karcinoma dojke pokazuju u terminalnim duktulima atipične hiperplastične nodule iz kojih zatim, tijekom vremena, proliferira tumor. Tablica 3 pokazuje razvoj mliječne žlijezde i pojavu hiperplastičnog nodula u štakora.

Tablica 3

RAZVOJ MLIJEČNE ŽLIJEZDE U ŠTAKORA

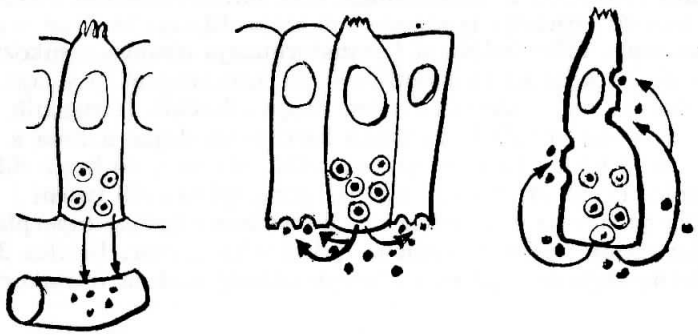


Iako imamo jasnu koncepciju o nastanku najčešćih hormon-ovisnih tumora u čovjeka (dojka, prostata, endometrij, ovarij), još uvijek postoji veliki raskorak u našem poznavanju učinaka hormona i stanja koja omogućuju kancerogenezu. Uz klasični endokrini model regulacije rasta i diferencijacije, veliki značaj ima parakrini i autokrini regulacijski model. Bitna značajka autokrine i parakrine regulacije je sekrecija staničnih i tkivnih čimbenika rasta (poticajnih i inhibitornih) koji moduliraju funkcije stanica na lokalnom nivou, što pokazuje shema na tablici 4.

Steroidni hormoni u ciljnim stanicama moduliraju ekspresiju pojedinih gena i protoonkogena. Zbog važnosti protoonkogena u regulaciji rasta i diferencijacije, zadnjih se godina istražuju interakcije steroidnih hormona i protoonkogena. Značajan nalaz je homolognost sekvencija estrogenskih i glukokortikosteroidnih citoplazmatskih receptora i produkta erb-A onkogena (3). (Tablica 5).

Tablica 4

DIJAGRAMSKI PRIKAZ ENDORINE, AUTOKRINE I PARAKRINE SEKRECIJE

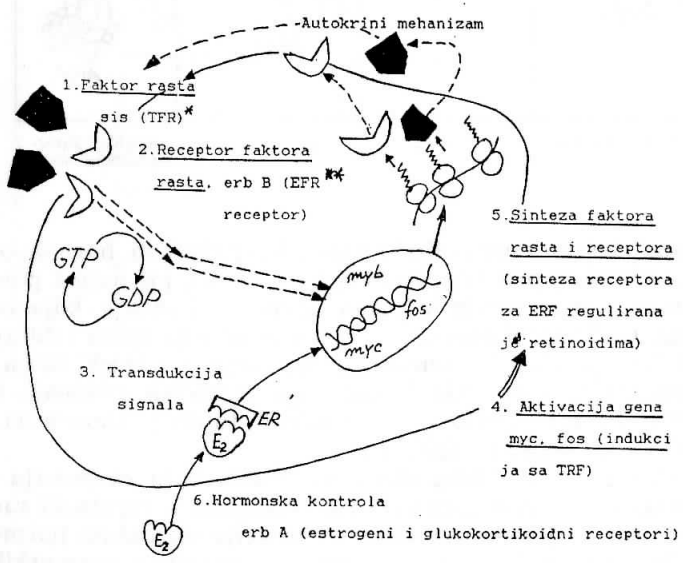


Endokrini Parakrini Autokrini

Na tablici 5 prikazana je shema poznatih učinaka staničnih onkogena koji potiču ekspresiju peptidnih faktora rasta u autokrinom smislu. Prikazana je i interakcija steroida i retinoida sa onkogenima i peptidnim faktorima rasta.

Tablica 5

INTERREAKCIJA STEROIDA I RETINOIDA SA ONKOGENIMA I PEPTIDNIM FAKTORIMA RASTA



* TFR — trombocitni faktor rasta
** EFR — epidermalni faktor rasta

MOGUĆNOSTI I REZULTATI ENDOKRINOLOŠKIH ISPITIVANJA KOD KARCINOMA DOJKE

Cilj je endokrinoloških ispitivanja da u bolesnika s nabrojenim hormon-ovisnim tumorima verificiraju hormonalne otklone, metaboličke promjene i druge faktore rizika. Modifikacijom ovih faktora, u još zdravih rizičnih skupina, postoji mogućnost prevencije hormon-ovisnih tumora (4). Endokrinološki zahvati koji modificiraju nutarnju hormonalnu okolinu putem ablativnih i aditivnih zahvata poznati su oko stotinjak godina i korisni su u liječenju uznapredovalog tumora dojke, odnosno prostate (5, 6).

Danas su endokrinološka istraživanja usmjerena na određivanje sekrecije i metabolizma svih poznatih regulatornih hormona dojke (estrogeni, progesteron, gonadotropini, prolaktin, inzulin, kortizol, hormon rasta, tiroksin) kao i na procjenu funkcionalnog stanja osovine hipotalamus-hipofiza-štitnjača, te osi hipotalamus-hipofiza-kora nadbubrežne žlijezde (KNŽ) (5, 7). U širem smislu, u endokrinološke pretrage možemo ubrojiti mjerenje membranskih receptora stanica dojke za polipeptidne hormone (hormon rasta, prolaktin, placentarni laktogen, inzulin) kao i membranskih receptora za čimbenike rasta. Tu možemo, također, ubrojiti i određivanje slobodnih citoplazmatskih receptora za estradiol, progesteron, kortizol i testosteron u stanicama karcinoma dojke. Saznanje o receptorima olakšava prognozu toka bolesti kao i optimalni izbor adjuvantne, odnosno palijativne hormonske terapije.

Tablica 6 pokazuje endokrinološke pretrage koje je moguće učiniti u bolesnica s karcinomom dojke.

Tablica 6

ENDOKRINOLOŠKE PRETRAGE U BOLESNICA S KARCINOMOM DOJKE

1. Hipotalamo-hipofizarno-ovarijalna os: GNRH, LH, FSH, estradiol (E_2), progesteron (P)
2. Hipotalamo-hipofizarno-adrenalna osovina CRF, ACTH, kortizol, androstendion, androsteron, dehidroepiandrosteron (DHEA)
3. Hipotalamo-hipofizarno-tiroidna osovina: TSH, T_3, T_4, rev. T_3, TRH test
4. Procjena periferne konverzije androstendiona u estron i estradiol u bolesnica s ovarijektomijom
5. Razina i dnevni ritam sekrecije prolaktina
6. Određivanje slobodnih frakcija steroidnih hormona

Endokrinološko-epidemiološke studije pokazale su kod karcinoma dojke dominantnu ulogu estrogena i prolaktina kao promotora tumorskog razvoja. Ota i sur. (7) našli su u žena s karcinomom dojke prije i nakon mastektomije, bez obzira na menopauzalni status, povećanu razinu neproteinski vezanog estradiola (NPVE), kao i estradiola vezanog na albumine. Cole i sur. (8) našli su isto tako u premenopauzalnih žena s karcinomom dojke povećane vrijednosti plazmatskog estradiola za 15 posto. To bi značilo da su žene s karcinomom dojke vjerojatno izložene višoj razini biološki raspoloživog estradiola. Sličan pomak u vezivanju estradiola na albumine i smanjeno vezivanje na SHBG (Sex Hormone Binding Globulin/globulin koji veže spolne hor-

mone) također je nađen i u zdravih adipoznih žena. U žena s karcinomom dojke je taj pomak konstantan, bez obzira na tjelesnu težinu odnosno masu masnog tkiva. Stoga ovaj nalaz u tih bolesnica omogućuje nutricione zahvate, to jest redukciju unosa masti i masnih kiselina. Za promociju karcinoma dojke važna je ne samo razina estrogena već i trajanje estrogene stimulacije. To potvrđuju epidemiološki podaci o ranoj menarhi i kasnoj menopauzi kao važnim faktorima rizika (1, 6, 7).

U zdravih kćeri žena oboljelih od karcinoma dojke nađene su povišene koncentracije estrogena i prolaktina u krvi i urinu. U njih je također utvrđena i povišena razina progesterona u krvi što Trichopulos i sur. (9) te Henderson i sur. (1) tumače većom učestalošću ovulacija u ispitivanih djevojaka.

Nadalje, Hill i sur. (10) su u žena s karcinomom dojke našli povišenu sekreciju prolaktina iz adenohipofize. Druge studije to međutim nisu potvrdile (11, 12), možda zato, jer nije praćen dnevni ritam sekrecije ovog hormona. U žena postmenopauzalne dobi liječenih rezerpinom nađene su visoke vrijednosti prolaktina slične onima u postpartalnom laktacijskom dobu (12). U njih nije sa sigurnošću utvrđen i povećan rizik nastanka karcinoma dojke. Međutim, početna epidemiološka ispitivanja Bostonske i Oxfordske skupine istraživača (13, 14, 15) sedamdesetih godina, pokazala su ipak veći rizik karcinoma dojke u onih žena koje su kroz duže vrijeme uzimale preparate rezerpina. Zato u preventivnom smislu, ove preparate treba davati s oprezom ženama s povećanim rizikom karcinoma dojke. Poznato je da je sekrecija prolaktina promijenjena i u hipotireozi. U žena s karcinomom dojke nađene su u serumu relativno povećane vrijednosti TSH uz uredne vrijednosti hormona štitnjače (16). Hormoni štitnjače reguliraju sintezu SHBG. U hipotireozi je njegova razina snižena pa je više estradiola vezano za albumine (17). Povećana biološka raspoloživost estradiola u tom slučaju mogla bi objasniti i veći relativni rizik karcinoma dojke u hipotireotičnih žena. Kunihiko i sur. našli su među 1810 bolesnica s Hashimoto-tiroiditisom 17 bolesnica s karcinomom dojke, što je pet puta više nego u kontrolnoj skupini (18). Stoga je kod svih oboljelih žena s karcinomom dojke, kao i u rizičnoj grupi, potrebno minuciozno ispitati funkciju štitnjače. U slučaju manifestne ili larvirane hipotireoze neophodna je supstituciona terapija hormonima štitnjače.

PRAKTIČNA PRIMJENA ENDOKRINOLOŠKIH SAZNANJA KOD KARCINOMA DOJKE

U bolesnica s ranim karcinomom dojke s malom vjerojatnošću udaljenih metastaza, važan je uvid u sva endokrinološka zbivanja koja su mogla a mogu i u daljnjem toku bolesti utjecati na ponašanje tumora. Prirodni tok bolesti pokazuje da žene s infiltracijom regionalnih limfonoda imaju visoki rizik udaljenih mikrometastaza, tako da se unazad trideset godina razvija koncept adjuvantne hormonske i adjuvantne kemoterapije. Prospektivne randomizirane studije o vrijednosti adjuvantne kemoterapije pokazale su produženje slobodnog intervala i duže preživljenje u skupini premenopauzalnih bolesnica (19). Međutim, u postmenopauzalnih bolesnica liječenih adjuvantnom kemoterapijom prednost u preživljavanju je minimalna (20—21). Nasuprot tome, adjuvantna hormonska terapija u postmenopauzalnih bolesnica rezultira najčešće značajnim produženjem slobodnog intervala i preživljenja. Osnovu adjuvantne endokrine terapije u postmenopauzi danas čini davanje antiestrogena tamoksifena (22—24). Nažalost, sjajne rezultate adjuvantne primjene tamoksifena u postmenopauzi ne prati ista djelotvornost tamoksifena u bolesnica s karcinomom dojke premenopauzalne dobi. Osim nekoliko prospektivnih randomiziranih evropskih studija o adjuvantnoj primjeni tamoksifena u premenopauzalnih bolesnica ovaj način liječenja u svijetu još nije prihvaćen (25—27). Iz tih evropskih studija vidljiv je ipak povoljan učinak tamoksifena u ovoj skupini bolesnica. Navedena nesuglasja bit će vjerojatno razjašnjena onda, kad budemo raspolagali konačnim rezultatima spomenutih prospektivnih studija. Do tada će u SAD, u premenopauzalnih bolesnica s karcinomom dojke adjuvantna kemoterapija i nadalje biti terapija izbora (27).

Manja djelotvornost adjuvantne primjene tamoksifena u premenopauzalnih bolesnica s karcinomom dojke nije objašnjena. Mogla bi se tumačiti očuvanom funkcijom i reaktivnošću ovarija u onih žena koje nisu iza mastektomije ovarijektomirane. Kod ovarijektomiranih bolesnica neuspjeh adjuvantne primjene tamoksifena možemo tumačiti sekrecijom prekurzora estrogena iz KNŽ i njihovom konverzijom u biološki aktivni estradiol u perifernim tkivima. Iz prilično rijetkih studija o reperkusijama tamoksifena na sekreciju hormona u zdravih i bolesnih žena, navodimo rad Shermana i sur. (28). Oni su u maloj skupini zdravih žena koje su uzimale tamoksifen našli održane regularne menzese sa bifazičnim temperaturama i klasičnom dinamikom estradiola, uz dvostruko veće vrijednosti estradiola od normalnih i dva do tri puta povišen progesteron u luteinskoj fazi. Vrijednosti LH i FSH bile su uobičajene. Nivo prolaktina nije bio promijenjen. U studiji o adjuvantnoj primjeni tamoksifena u Christie Hospital i Holt Radium Institute (21), samo je 17% žena premenopauzalne dobi tretiranih tamoksifenom imalo trajnu amenoreju dok su u 52% bolesnica menzesi bili održani. Prateći funkciju štitnjače, uz istovremeno uzimanje tamoksifena, Gordon je našao porast tiroksina (T_4) i trijodtironina (T_3) kao i nosača tiroksina (TBG-Thyroxine Binding Globulin) u postmenopauzalnih bolesnica s karcinomom dojke (29). Ova zapažanja o povišenoj razini estrogena u premenopauzalnih žena, kao i promjene u sekreciji hormona štitnjače u postmenopauzalnih osoba tretiranih tamoksifenom, zahtijevaju daljnja endokrinološka istraživanja sekrecije, transporta i metabolizma estrogena, progesterona i njihovih nosača, kao i procjenu funkcije štitnjače (30—32).

ZAKLJUČAK

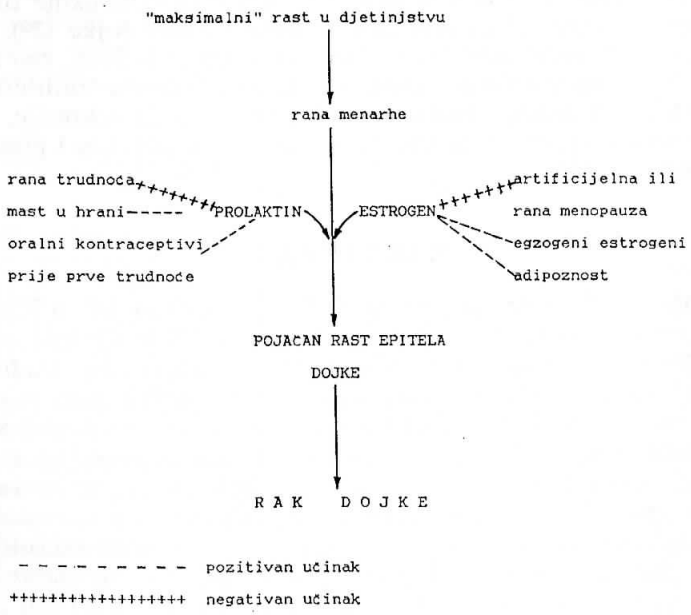
Današnje je liječenje karcinoma dojke poboljšano zahvaljujući manjoj masi primarnog tumora u većeg broja bolesnica. Zahvaljujući endokrinološkim istraživanjima dobro su definirani faktori rizika: prva trudnoća u kasnoj životnoj dobi, porodična anamneza karcinoma dojke, rana menarha i kasna menopauza, adipozitet, povećan unos masne hrane uz smanjeni unos povrća i retinoida i benigna bolest dojke s displastičnim promjenama terminalnih duktula. U ugroženim skupinama i kod bolesnica s ranim karcinomom dojke bilo bi potrebno studirati i sekreciju prolaktina i savjetovati oprez pri uzimanju lijekova (trankvilizatori, antidepresivi, alkaloidi rauvolije, alfametil dopa). U bolesnica s ranim karcinomom dojke treba uz nabrojene faktore misliti i na poremećaje u funkciji štitnjače i sve zajedno uzeti u obzir pri

planiranju daljnjeg terapijskog postupka. Samo određivanje citosolskih receptora za E_2 i P (ER i PR), kao i u slučaju visokog rizika primjene adjuvantne kemo- ili hormonalne ili kemo-hormonalne terapije nije dovoljno. Uvijek treba uzeti u obzir navedene rizične faktore koji su i nakon mastektomije i dalje prisutni i nastavljaju svoj pogubni učinak na udaljene mikrometastaze tumora.

Današnja saznanja o prirodnom toku karcinoma dojke prihvaćaju dugu indukcijsku i promocijsku fazu bolesti i mada nismo sigurni do kojeg su stupnja inicijacijske promjene reverzibilne, potrebno je u svih ugroženih žena u što većoj mjeri reducirati poznate faktore rizika. S druge strane ovaj koncept sadrži u sebi i pojam sistemne bolesti od samog početka što znači da treba i dalje tražiti bolje oblike adjuvantne terapije karcinoma dojke. To će biti moguće usvajanjem novih saznanja molekularne biologije o interakciji hormona, faktora rasta i onkogena primjenom modifikatora faktora rasta (retinoidi) u kombinaciji s antiestrogenima odnosno citostaticima. Ova koncepcija ne isključuje stari Halstedtov pristup o primarnoj lokalnoj bolesti, jer niz objektivnih studija pokazuje da samo dobra lokalna kirurška i eventualno radiološka terapija predstavlja osnovni uvjet za dugo preživljenje bolesnica s karcinomom dojke. Vidljivo je također da se neke faktore može mijenjati što daje nade u manju incidenciju karcinoma dojke od kojeg u razvijenim zemljama oboljeva svaka četrnaesta žena.

Tablica 7

ČINIOCI KOJI UTJEČU NA NIVO ESTROGENA I PROLAKTINA I NA PROLIFERACIJU TKIVA DOJKE



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SAŽETAK

ZNAČENJE ENDOKRINOLOŠKIH PRETRAGA U ŽENA S RANIM KARCINOMOM DOJKE

Faktorima rizika za pojavu karcinoma dojke u žena smatraju se danas rana menarha, prva trudnoća u dobi iznad 30 godina života, familijarna pojava karcinoma dojke, pojava fibrocistične bolesti dojke s displazijom terminalnih kanalića, adipozitet, dijeta bogata mastima i siromašna povrćem i retinoidima. U žena sa spomenutim faktorima rizika kao i u onih s verificiranim ranim karcinomom dojke potrebno je utvrditi i sekreciju prolaktina. U žena u kojih se nađe hiperprolaktinemija potreban je oprez pri medikaciji psihofarmacima, alkaloidima rauvolfije, metildopom, antagonistima kalcija i insulinom.

U žena s ranim karcinomom dojke korisno je učiniti funkcionalne testove štitnjače zbog potencijalnih učinaka TRH na sekreciju prolaktina a i stoga što hipometabolizam ili hipermetabolizam mogu direktno utjecati na brzinu rasta tumorskih stanica. Određivanje receptora estradiola i progesterona u tumorskim stanicama za terapiju i prognozu tumora dojke je od neprocijenjive važnosti; no samo uz poštovanje gore nabrojenih faktora rizika koji su i dalje prisutni nakon kirurškog zahvata, odnosno samo uz eliminaciju odnosno redukciju faktora rizika možemo u žena s ranim karcinomom dojke očekivati optimalne učinke liječenja.

SUMMARY

THE ROLE OF HORMONAL ASSESSMENTS IN EARLY BREAST CANCERS

Accepted risk factors for breast cancer in females are: age over 30 years at first birth, family history of premenopausal bilateral breast cancer, early menarche, history of fibrocystic disease with dysplasia of terminal ducts, obesity, and diet rich in fats and defitient in vegetables and retinoids. Women with these risk factors and those with already diagnosed early breast cancer need prolactin secretion assessment. In the case of hyperprolactinemic women drugs such as psychopharmaca, rauwolfia alcaloides, alphametyl dopa, calcium antagonists and insulin should be avoided.

In women with early breast cancer the assessment of the functional status of the thyroid gland is also advisable because of the potential influence of TRH on prolactin release and hyper or hypomethabolism can directly influence the growth of tumor cells. Estradiol and progesteron receptor assessment in breast cancer cells plays a dominant role in the prognostic and therapeutic (chemotherapy, hormonal therapy) approach to breast cancer treatment but if the above mentioned risk factors are present and after mastectomy this can influence tumor promotion and/or regression, only a combination of a rational approach to ER and PR and elimination and diminution of persistent risk factors can yield optimal treatment results.

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ORAL HEALTH CONDITIONS AND TREATMENT NEEDS FOR YOUTH FROM DIFFERENT REGIONS OF YUGOSLAVIA

INTRODUCTION

In the vast majority of cases, oral health problems are the result of caries and periodontal diseases.

The pattern of caries prevalence has changed enormously. The level of caries has fallen in places with traditionally high prevalence, while it has risen in developing cities (WHO 1982, FDI 1984). The reasons for these changes have not yet been established, but are most probably connected with a broader usage of fluorides and a higher sugar consumption by persons without fluoride protection. These changes have brought about various problems that need different solutions (WHO, 1984.).

The prevalence of periodontal diseases is unknown, but existing data suggest that they are omnipresent in undeveloped and developing countries, and that the majority of adults in developed countries suffer from them (Page 1985.). Therefore these diseases are a major public health problem all over the world.

In the last two decades great efforts have been made in investigating these diseases, trying to find out better treatment and prevention methods. Enormous advances have been made in our understanding of the etiology and development periodontal diseases in the last decade, which makes obligatory the control and active prevention of these diseases (Ainamo 1984., Colin 1986, Künce 1984, Burt 1984.).

Although all etiological factors are not completely clear, it is a well established fact that caries and periodontal diseases can be effectively kept at bay, if not completely eliminated using different preventive strategies that will more and more depend on public health measures and individual efforts of non-professional and professional dental health workers (Preventive Dental Services, 1980.).

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SAMPLE AND METHODS

An epidemiologic study of young participants of the Sava 84/85 Youth Work Camp has been carried out with the goal to register the prevalence of periodontal diseases, caries, dentofacial anomalies and oral mucosa diseases. The participants in this project came from different regions of Yugoslavia; all republics and autonomous regions are represented (Appendix 2). The sample, on the other hand, is not representative of the geographic and age distribution of youth in Yugoslavia. Still, we think that the results from sample can provide an important insight into oral health conditions in youth 15—30 years old. Approximately 2000 persons were examined. Shown are results of examination of approximately 1900 persons divided in age groups of 12,5——19; 20—24; 25—29; and 30—34 years. The age groups 12 and 30—34 were too small for a decent statistical analysis and were therefore excluded from the study. The age profile of the sample is shown in Figure 1.1.

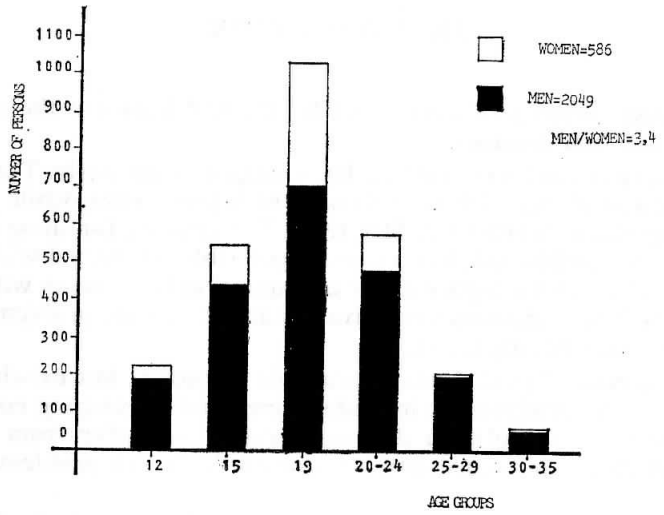

FIGURE 1.1. SIZE PROFILE OF THE AGE GROUPS CONSIDERED IN THE STUDY

For the assessment of oral health conditions, standard methods and indices recommended by WHO were used (WHO basic oral health assessment form with CPITN 1982). For assessment of periodontal diseases, periodontal indices for bleeding from gingival sulci, dental calculus, and shallow and deep periodontal pockets were used. The index was defined as the mean number of sextants per person in which the given condition can be found. Dental caries condition was measured using the DMF index, i.e. the mean number of teeth per person showing caries, filling or extraction. Dentofacial anomalies were classified according to treatment needs. The prevalence of the diseases was measured by the percentage of persons that had a given disease condition on at least one sextant (periodontal index) or tooth (caries, filling, extraction).

During the study it became clear that some examiners differ in their assessment of periodontal disease classification and progression by more than 10%. Therefore, some correction and reevaluation of data, specially those concerning periodontal pocket depth, were made so that the errors of the mean values of evaluation would not recede from the expected standard deviations calculated on the basis of earlier pilot studies.

RESULTS

1. Periodontal Diseases

The periodontal tissue condition of the examined population was determined using the CPITN-index. Bleeding from the gingival sulcus was registered as the earliest sign of gingival inflammatory changes, further calculus layers, shallow periodontal pockets measuring 4—5 mm, and periodontal changes including deep periodontal pockets (6 mm and more), as shown in Figure 2.1. The values and their standard deviations are given in Table 2.1. Gingival sulcus bleeding can be found in an average of 4—5 sextants per person. Calculus is present in 2 to 3 sextants, and periodontal pockets in approximately 30—40% of sextants (shallow pockets). The measurement errors (a half span for a 95% reliability interval), for the gingival sulcus bleeding index and

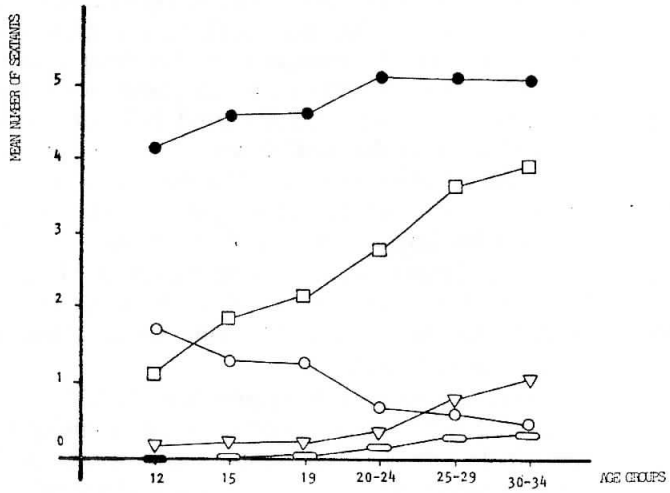

FIGURE 2.1. PERIODONTAL INDICES VS. AGE GROUPS (CPITN)

○ H=HEALTHY ● BL=BLEEDING
□ CA= CALCULUS ▽ P4=POCKETS 4-5 mm
⬭ P6=POCKETS 6 mm

Table 2.1.

PERIODONTAL INDICES FOR YOUNG PERSONS FROM DIFFERENT PARTS OF YUGOSLAVIA. STANDARD DEVIATIONS OF THE MEAN ARE GIVEN IN PARENTHESES

Age	*Sample N*	*Healthy sextants*	*Bleeding*	*Calculus*	*Pockets 4 mm*	*Pockets 6 mm*
12	222	1,79	4,14	1,17	0,15	0,00
		0,14	0,14	0,12	0,03	0,00
15	548	1,32	4,65	1,99	0,28	0,01
		0,08	0,08	0,09	0,03	0,00
19	1027	1,35	4,60	2,17	0,27	0,02
		0,06	0,06	0,06	0,02	0,00
20—24	569	0,75	5,11	2,83	0,46	0,68
		0,06	0,64	0,09	0,04	0,02
25—29	214	0,62	5,10	3,63	0,76	0,47
		0,08	0,98	0,14	0,09	0,02
30—34	55	0,50	5,12	3,92	1,16	0,25
		0,15	0,17	0,26	0,23	0,09

calculus, are between 2.5% and 9% for periodontal pockets between 17% and 75%. The latter values are the result of the relatively small number of persons showing signs of periodontitis.

It is useful to compare these values with values for SR Croatia. For age groups from 15 to 29 years, this sample has either significantly *lower* or approximately the same values of periodontal indices. The differences are not great, being between 0.5 and 1.0. An exception is the deep pocket index for the age group 15—19. The youth participating in these work projects have significantly higher values of this index (approximately 7 times greater). According to the reevaluation of data on the participants of the youth work projects' this difference is the result of examiners' criteria deviation. The examination was made by a larger group of examiners, and the differences in their assessmet of periodontal conditions had not been tested before.

The prevalence of periodontal diseases in participants of the YWA is given in Figure 2.2. The prevalence was measured as the percentage of persons having the periodontal tissue changes in at least one sextant. More severe changes imply the existence of lesser ones.

Gingivitis is, for example, present in approximately 73% to 87% of the YWA participants (Table 2.2.). Periodontitis of medium intensity with pockets 4—5 mm deep is found in 13—29% of participants and severe periodontal disease is found in approximately 1—4% of the YWA participants.

Treatment needs are shown in Table 2.3. and Figure 2.3. as the percentage of participants that need dental treatment because of periodontal tissue changes in at least one sextant. The treatment is determined according to the most severe periodontal condition of the examined person.

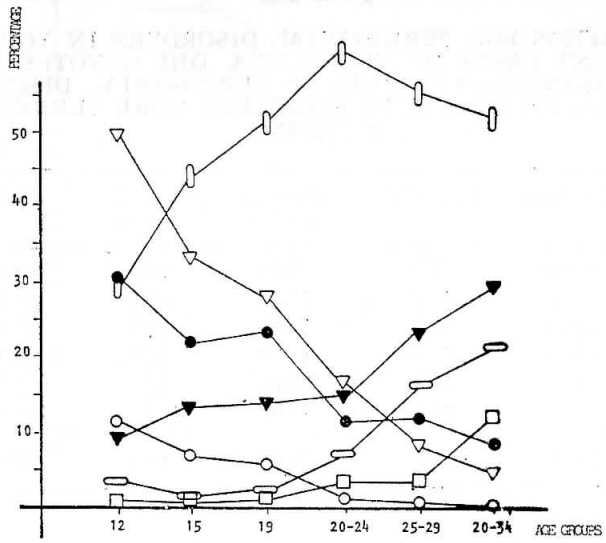

FIGURE 2.2. PREVALENCE OF PERIODONTAL DISEASE VS. AGE GROUPS IN YOUTH OF YUGOSLAVIA

○ =HEALTH ▽BL=BLEEDING ▯CA=CALCULUS
▼P4=POCKETS 4-5 mm □P6=POCKETS 6 mm
⊂⊃ XPO=EXCLUDED ● HEALTHY=3 SEXTANTS

The results show that 94% of the youth need health education or instruction concerning oral hygiene measures. Prophylactic therapeutic measures or calculus cleaning are needed by approxmately 70% of YWA participants from all parts of Yugoslavia. Special periodontological treatment is needed by approximately 2% of the youth.

Table 2.2.

PREVALENCE OF PERIODONTAL DISORDERS IN YOUNG PERSONS FROM DIFFERENT PARTS OF YUGOSLAVIA

Age	*Healthy*	*Bleeding*	*Calculus*	*Pockets 4 mm*	*Pockets 6 mm*
12	11.26	50.00	29.28	9.00	0.45
15	7.29	33.39	44.71	13.68	0.72
19	5.93	26.23	51.51	13.04	1.07
20—24	1.75	16.16	61.86	16.87	3.18
25—29	0.93	8.87	59.35	27.10	3.73
30—34	0.00	5.45	52.73	29.09	12.72
Total	5.23	26.49	51.12	15.14	1.85

Table 2..3.

TREATMENT NEEDS FOR PERIODONTAL DISORDERS IN YOUNG PERSONS FROM DIFFERENT PARTS OF YUGOSLAVIA. OHI DENOTES PERCENTAGE OF PERSONS SHOWING ANY KIND OF PERIODONTAL DISORDER, WHILE PROPH DENOTES PERSONS WITH DISORDERS MORE SERIOUS THAN JUST BLEEDING

Age	*Bleeding*	*Calculus*	*Pockets 4 mm*	*Pockets 6 mm*	*OHI*	*Proph*
12	50.00	29.28	9.00	0.45	88.74	38.74
15	33.39	44.71	13.68	0.72	92.82	59.12
19	26.23	51.51	13.04	1.07	93.87	65.63
20—24	16.16	61.86	16.87	3.18	98.07	81.90
25—29	8.87	59.35	27.10	3.73	99.07	90.19
30—34	5.45	52.73	29.09	12.72	100.00	94.55
Total	26.49	51.12	15.14	1.85	94.61	68.12

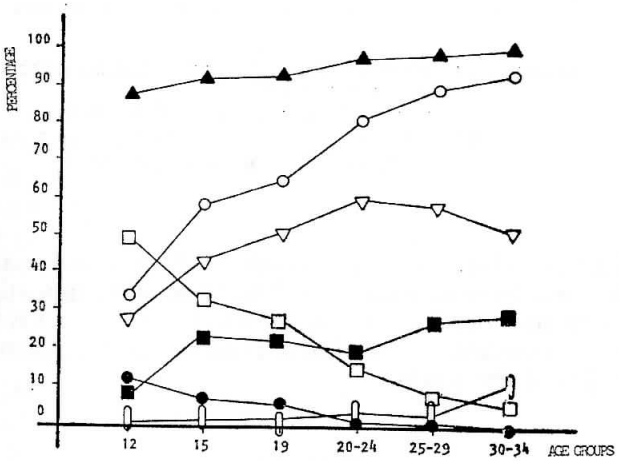

FIGURE 2.3. TREATMENT NEEDS FOR PERIODONTAL DISORDERS IN YOUNG PERSONS FROM DIFFERENT PARTS OF YUGOSLAVIA
●=HEALTHY □BL=BLEEDING ▽CA=CALCULUS
■=POCKET 4-5mm ▯=POCKET 6 mm— COMPLETE SPECIALIST CARE
▲=OHI — EDUCATION
○=PROPHYLACTIC

2. *Caries*

The dental status is shown by the DMF index in Figure 3.1. The values of the index components: caries, filling and missing teeth and standard deviations of the mean are given in Table 3.1. The DMF index in the 15—29 years old group is 10: 6 teeth have caries, 2 are filled, and 2 have been extracted. The values are either significantly higher or lower than the average for Croatia in age groups 15, 19, and 20—24.

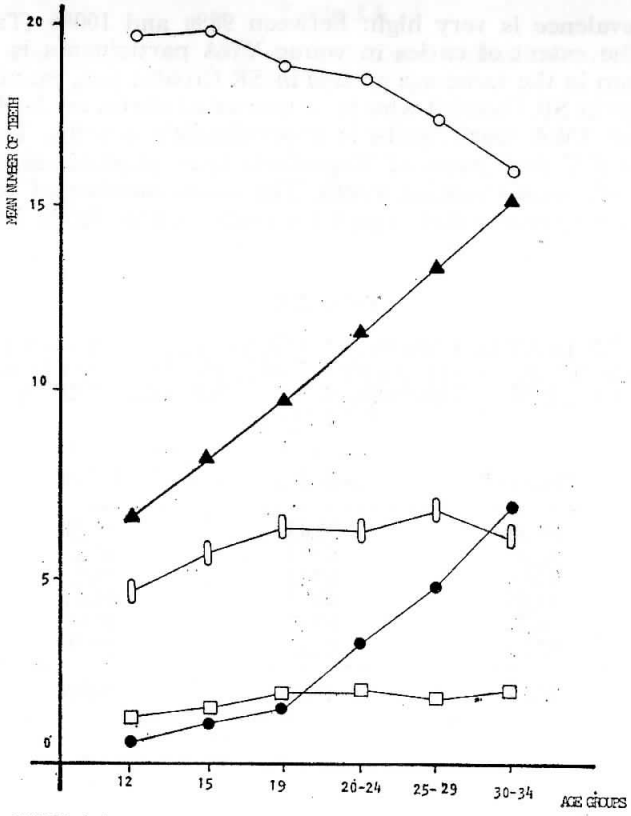

FIGURE 3.1. DMF INDEX VS AGE GROUPS
○=HEALTH ▯D= DECAYED ●M=MISSING
□F=FILLED ▲DMF= D+M+F

Table 3.1.

DMF INDICES FOR YOUNG PERSONS FROM DIFFERENT PARTS OF YUGOSLAVIA. STANDARD DEVIATIONS ARE GIVEN IN PARENTHESES

Age	*Decayed*	*Missing*	*Filled*	*DMF*
12	4.62	0.65	1.32	6.59
	(0.24)	(0.85)	(0.19)	(0.28)
15	5.55	1.11	1.57	8.24
	(0.12)	(0.64)	(0.11)	(0.19)
19	6.31	1.54	1.96	9.84
	(0.13)	(0.57)	(0.97)	(0.15)
20—24	6.24	3.37	1.99	11.60
	(0.18)	(0.14)	(0.13)	(0.22)
25—29	6.62	4.84	2.00	13.36
	(0.30)	(0.28)	(0.20)	(0.38)
30—34	6.12	6.94	1.88	15.12
	(0.53)	(0.64)	(0.41)	(0.68)

Caries prevalence is very high: between 98% and 100% (Table 3.2. and Figure 3.2.). The extent of caries in young YWA participants is the same or even higher than in the same age groups in SR Croatia (i.e., examined persons of the same age in SR Croatia) who have untreated caries on 3—4 teeth, while the average for YWA participants is approximately 6 teeth. The YWA participants from different parts of Yugoslavia have approximately two times fewer filled teeth than Croatian youth. The mean number of teeth with secondary caries is approximately equal for both groups (Table 3.3.).

Table 3.2.

PREVALENCE OF DENTAL CARIES IN YOUNG PERSONS FROM DIFFERENT PARTS OF YUGOSLAVIA. PREVALENCE IS EXPRESSED AS THE PERCENTAGE OF PERSONS SHOWING AT LEAST ONE TOOTH IN A GIVEN DMF STATE

Age	*Decayed*	*Missing*	*Filled*	*DMF*
12	89.64	32.43	48.20	97.35
15	92.34	62.92	44.69	97.63
19	93.48	62.41	46.25	98.15
20—24	93.85	80.32	46.40	99.65
25—29	95.33	89.25	47.66	100.00
30—34	92.73	92.73	50.91	100.00
Total	93.13	64.59	40.38	98.52

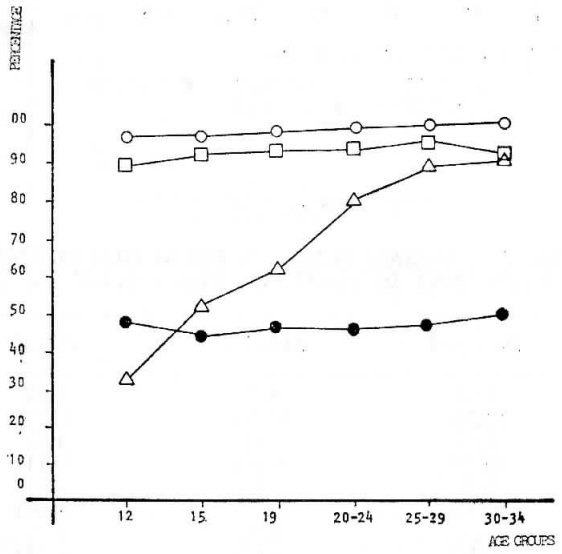

FIGURE 3.2. PREVALENCE OF DENTAL CARIES FOR YOUNG PERSONS FROM DIFFERENT PARTS OF YUGOSLAVIA

○ =DMF △ M=MISSING □ D=DECAYED
● F=FILLED

Table 3.3.

DMF FINE STRUCTURE IN YOUNG PERSONS FROM DIFFERENT PARTS OF YUGOSLAVIA

Categories are: Caries (untreated), filled tooth (no caries), filled tooth with primary caries (FPC), filled tooth with secondary caries (FSC), permanent tooth missing due to caries (PTMC), permanent tooth missing for other reasons (PTMOR), unerupted tooth, tooth excluded from analysis. The extent is measured by the average number of teeth affected per person.

Age	*Caries*	*Filled*	*FPC*	*FSC*	*PTMC*	*PTMOR*	*Uner-uped*	*Ex-cluded*	*Caries-0*
12	4.17	1.32	0.76	0.36	0.64	0.00	5.72	0.02	19.65
15	5.08	1.97	0.51	0.41	1.11	0.003	3.99	0.22	19.74
19	5.70	1.96	0.65	0.54	1.54	0.02	3.36	0.22	18.79
20—24	5.77	2.01	0.58	0.40	3.35	0.02	1.84	0.63	18.47
25—29	6.11	1.88	0.51	0.45	4.36	0.08	1.30	0.10	17.23
30—34	5.69	2.05	0.18	0.41	5.25	1.69	0.73	0.21	15.93
Total	5.49	1.83	0.06	0.46	2.11	0.05	3.14	0.42	18.80

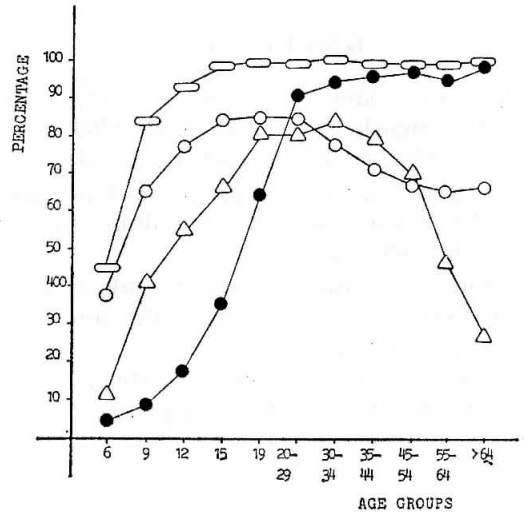

FIGURE 3.3.DMF INDEX VS AGE GROUPS

▭ =DMF ○ D=DECAYED
● M=MISSING △ F=FILLED

3. *Dentofacial anomalies*

The prevalence of dentofacial anomalies for the population sample of young YWA participants is shown in Table 4. 1. On the average, dentofacial

anomalies have been found in 34% of cases, with 8% needing treatment. For comparison, orthodontic anomalies prevalence for the same age groups in SR Croatia is 36%, and 20% of examined persons are in need of treatment.

Table 4.1.

DENTOFACIAL ANOMALIES SHOWN BY YOUNG PERSONS FROM DIFFERENT PARTS OF YUGOSLAVIA. »PRESENT« DENOTES THE PERCENTAGE OF PERSONS EXHIBITING A DENTOFACIAL ANOMALY, »TRETMENT«, DENOTES THE PERCENTAGE NEEDING TREATMENT FOR THE OBSERVED ANOMALY.

Age	*Sample*	*Present*	*Treatment*
12	222	21.48	10.81
15	548	35.91	11.84
19	1027	34.07	9.54
20—24	569	34.16	8.43
25—29	214	27.07	5.60
30—34	55	20.93	1.81

CONCLUSION

Results of epidemiologic studies dealing with the oral health of youth from different parts of Yugoslavia 1984/85 Sava YWP participants) show a very high frequency of caries, gingivitis, and dentofacial anomalies.

It is possible to organize a realistic program of preventive measures based on these results. In performing and maintaining these measures, health education is of utmost importance.

Evaluation of planned measures enables the planning of oral health improvement and the assessment of achieved results, and modifications of existing active prevention programs.

Health education and the performance prophylactic measures, like calculus celaning, should be the right of all young people in our country, because the most important reason for early teeth extraction is the absence of early diagnosis and prevention in dental practice.

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APPENDIX 2

1984/85 SAVA YOUTH WORK PROJRCT (YWP)

	Number of examined persons
CROATIA	
Koprivnica	71
Osijek	49
Bjelovar	40
Belišće	67
Darda	58
Split	30
Benkovac	62
Metković	52
Zabok	67
Kutina	25
Zagreb	112
	633
BOSNIA AND HERCEGOVINA	
Sarajevo	8
Banja Luka	12
Stolac	41
Trebinje	53
Živinice	61
Brčko	71
Ugljevik	77
Bugojno	26
	349
MONTENEGRO	
Titograd	84
MACEDONIA	
Štip	19
Skopje	32
Kumanovo	110
	161
SERBIA	
Beograd	289
Lazarevac	56
Zaječar	19
Bor	72
Leskovac	70
Lučani	53
Požarevac	56

	Number of examined persons
Velika Plana	62
Kosjerići	73
Titovo Užice	68
Požega	49
	928
KOSOVO	
Priština	166
Peć	53
	219
VOJVODINA	
Subotica	9
Zrenjanin	68
Novi Sad	128
Ruma	48
Vršac	31
	248
SFR YUGOSLAVIA	
Jugoslavija (mješana brigada)	188
FOREIGNERS	
UDSSR	13
France	22
	35
TOTAL EXAMINED	2 881

SAŽETAK

Učestalost oralnih bolesti značajan je zdravstveni problem u svijetu i u nas. Danas pouzdano znamo da je prevencija oralnih bolesti mnogo uspješnija od liječenja. Djelotvorne mjere prevencije značajno smanjuju prevalenciju zubnog karijesa, a i bolesti parodonta. To pokazuju rezultati brojnih istraživanja u različitim zemljama.

Za planiranje realnih programa sustavne prevencije neophodna je informiranost o stanju oralnog zdravlja na osnovi bazičnih orijentacijskih podataka, što je i bio cilj ovog istraživanja.

Epidemiološkim istraživanjem obuhvatili smo prevalenciju parodontnih bolesti, karijesa, dentofacijalnih anomalija i bolesti sluznice usne šupljine u mladih, koji su učestvovali na radnim akcijama (ORA) Sava 1984/85. iz različitih krajeva naše zemlje. Zastupane su bile sve republike i pokrajine. Starost ispitanika se kretala od 15—30 godina. Ukupan broj ispitanika iznosio je 2.000 osoba. Za procjenu stanja oralnog zdravlja koristili smo standarde Svjetske zdravstvene organizacije.

Dobiveni rezultati pokazuju da je učestalost oralnih bolesti u mladih iz svih krajeva Jugoslavije veoma visoka. Gingivitis se mogao uočiti u 73—87% mladih, parodontitis srednjeg intenziteta s džepovima dubokim 4—5 mm registriran je u 13—24% ispitivanih učesnika radnih akcija, a uznapredovali parodontitis dubine džepova 6 i više mm registriran je u 1—4% mladih ovisno o razlici u godinama starosti.

DMF indeks mladih iz različitih krajeva Jugoslavije u dobi od 15—30 godina u prosjeku je bio 10—14, od čega 7 zubi ima karijes, 2 zuba imaju ispun, a 1—5 zubi je već izvađeno. Prevalencija karijesa (DMF) iznosila je 94%.Dentofacijalne anomalije u prosjeku su bile registrirane u 34% osoba.

Mjere zdravstvenog odgoja i upućivanja u dobro održavanje oralne higijene treba 94% mladih, profilaktičke postupke kojima bi se zaustavila progresija parodontnih bolesti treba provesti u 70% mladih stanovnika Jugoslavije, a specijalistički tretman u 2,5% ispitivanih osoba.

Da bi smanjili gubitak zubi i posljedice koje zbog toga nastaju u odrasloj dobi, neophodno je planirati sistem stomatološke zaštite, uključujući u mjere permanentne zaštite primarnu stomatološku prevenciju, te evaluaciju i kontrolu uspješnosti programa u skladu sa suvremenim dostignućima prevencije i terapije veoma učestalih bolesti usne šupljine.

SUMMARY

In order to assess the prevalence of periodontal diseases, dental caries and dentofacial anomalies, as well as of diseases of the mucous membrane of the oral cavity, we carried out an epidemiologic investigation into the oral health of the participants of the 1984/85 the Sava Youth Work Brigades, who came from all over Yugoslavia.. All the republics and regions of the country were represented. The total of about 2,800 young people aged 15—30 yrs. were examined. To evaluate the state of oral health we used criteria and methods recommended by the World Health Organization.

Results show that among the young people from different parts of the country, the frequency of oral diseases is very high. Gingivitis could be observed in 73—87% of participants, periodontitis with pockets 4—5 mm deep was recorded in 13—24% of the examines and advanced peridontal disease with pockets deeper than 6 mm in 1—4%, dependent on age differences.

The DMF index in ages 15—30 years is 10—14 i.e. 7 teeth on the average have caries, 2 are filled, and 1—5 extracted. Caries are found in up to 94% of individuals. Dentofacial anomalies have been recorded in an average of 34% of the examinees.

The measures of health education and instruction in hygienics of the oral cavity are needed in 94% of young people, prophylactic treatment measures to stop the progression of peridontal disease should be carried out in 70% and the specialistic periodontal therapy in 2,5% of the examines.

In order to reduce tooth loss and the consequences thereof, especially in adult age, it is indispensable to also include organized measures of primary prevention in stomatological protection, and evaluation and control of the efficiency of particular programs of protection against the most frequent diseases of modern man.

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PRINCIPI LIJEČENJA RANOG RAKA DOJKE U KLINIČKOM BOLNIČKOM CENTRU RIJEKA

UVOD

Danas u kliničkoj onkologiji postoji već prilično usklađen i jedinstven pristup u dijagnostici i terapiji većine malignih tumora (De Vita 1982). Tako su se prije tri decenije najprije u svijetu, a potom i u nas počele sve češće uočavati tendencije za primjenu modificiranih, poštednih kirurških zahvata kod ranog raka dojke (Kučišec 1980).

Na X internacionalnom onkološkom kongresu u Houstonu 1970. godine bilo je više pobornika manje mutilirajućih zahvata, čak i samo obične tumerektomije (Zbornik radova kongresa 1970). Ubrzo smo i u Rijeci započeli s primjenom modificirane mastektomije, kao i drugih ograničenih zahvata, pa se već niz godina kod ranog raka dojke više ne izvode radikalne Halstedove mastektomije (Pavlović 1983).

Sve do šezdesetih godina kod operabilnog raka dojke uz radikalnu mastektomiju nismo primjenjivali rutinsku postoperativnu radioterapiju. Indikacije operabilnosti bile su prilično široke, ali u tom vremenu jedino moguće i najdjelotvornije riješenje. Međutim, uz brojne slučajeve udaljenih metastaza bio je prisutan i veliki broj bolesnica sa proširenim lokalnim recidivima gdje smo mogli malo učiniti i pomoći.

Iz tih razloga već od 1960. godine počeli smo upotrebljavati rendgensku, a od 1965. godine pa sve do danas postoperativnu telekobaltnu terapiju (Pavlović 1971). Veoma brzo smo smanjili lokoregionalne recidive za oko 30% što smo u to vrijeme smatrali velikim uspjehom, pogotovo jer smo usavršili metodu zračenja tangencijalnim poljima uz individualnu dozimetriju.

OSNOVNI PRINCIPI LIJEČENJA RANOG RAKA DOJKE

Naš pristup i postupke u liječenju svih stadija karcinoma dojke usklađivali smo prema općenito prihvaćenim stanovištima i principima rada sličnih ustanova u svijetu, s izvjesnim manjim ličnim promjenama.

Stoga smo reducirane operativne zahvate prihvatili sa velikim entuzijazmom, jer smo ih mogli provoditi u kombinaciji sa preciznom i efikasnom postoperativnom radioterapijom (Pavlović 1975.).

Manje opsežne operacije kod sve učestalijeg raka dojke u Rijeci prihvatili smo i zbog toga što su i same žene, kako fizički tako i psihički, znatno lakše podnosile te zahvate. Istovremeno, i eksperimentalna zaostala tumorska žarišta, pogotovo mikro depoziti u operativnom području, se mogu potpuno uništiti sa dozom zračenja od 4500—5000 cGy. Zbog toga smo rutinski primjenjivali dodatno zračenje nakon operacije.

Nismo pobornici sasvim ograničene konzervativne kirurgije, odnosno malih zahvata, osobito nakon otkrića više onkologa (Galager, Martin, Urban 1978) da oko 50% slučajeva sa ranim stadijima raka dojke nastaju multicentrično, tj. da imaju multiple in situ tumore u cijeloj dojci. U slučajevima gdje smo išli na tako male zahvate redovito smo dodavali agresivno postoperativno zračenje.

To su bili naši osnovni principi u liječenju koje smo tijekom posljednje tri decenije sprovodili kroz timski rad. Timska suradnja kirurga i radioterapeuta sa većim ili manjim intenzitetom uhodana je i usklađena a nadopunjavana je učešćem patologa, radiologa, citologa te posljednjih godina i ultrazvučnih dijagnostičara.

Svakako treba naglasiti da danas u kliničkom bolničkom centru u Rijeci postoji jedinstvena kirurška i onkološka služba za dojku.

PROTOKOL DIJAGNOSTIKE I TERAPIJE

Principi liječenja odnosno razrađeni protokol za dijagnostičku obradu i metode liječenja raka dojke prihvačen je od cijelog tima uz povremene i dogovorne izmjene (Longhino 1981).

Kod ranog raka dojke od dijagnostičkih pregleda i pretraga sprovodimo slijedeće:

I Povijest bolesti — notiramo sve riziko-faktore. Prominentni faktori su slijedeći: dob, spol, obiteljska anamneza, broj poroda i mastopatije. Od glavnih faktora saznajemo o produženoj menstrualnoj aktivnosti, o uzimanju estrogena kod rotkinja, te eventualnom postojanju diabetesa melitusa, obesitasa ili hipertenzije. Konačno uzimamo podatke o mogućim riziko — faktorima (izloženost zračenju, virusima i traumi), te o svim relevantnim podacima u vezi sa sadašnjim oboljenjem odnosno o svim detaljima tumorskog procesa.

II Klinički pregled — opći, a posebno veoma precizan i detaljan lokalno — regionalni pregled dojka.

III kompletna laboratorijska obrada krvnih, jetrenih i bubrežnih nalaza i pretraga.

IV Mamografija i ultrazvučna pretraga dojka.

V Radiološki pregledi pluća, lubanje, kičme i zdjelice, a ukoliko je nešto sumnjivo vrši se i skan kostiju. Slično je i za jetru koja se pregledava scintigrafski ili UZ samo ako su promjenjeni laboratorijski testovi. Scintigrafske pretrage kostiju i jetre ne smatramo indiciranim u rutinskoj obradi ranih stadija.

VI Klinička klasifikacija tumora po stadijima prema TNM sistemu.

VII Patohistološke pretrage tumora su:

a) citološke pretrage s aspiracionim uzorcima ili needle biopsijom. Sumnjiv ili negativni nalaz ne isključuje malignom, a pozitivni nalaz nije indikacija za operativni zahvat nego tek u slučaju kada je potvrđen biopsijom.

b) Kirurška biopsija koja se u većini slučajeva provodi kao intraoperativna biopsija, a uvedena je rutinski prije dvadesetak godina.

c) Ovdje uključujemo i intraoperativno uzimanje uzoraka tumora za određivanje estrogenskih i progesteronskih receptora. Pretraga je uvedena prije 5—6 godina.

Prema našem protokolu terapiju ranog raka dojke sprovodimo prema kriterijima TNM klasifikacije za slijedeće skupine:

Skupina I

TIS — obuhvaća neinvazivni intraduktalni, lobularni ili na bradavicu ograničeni Paget karcinom. Osnovni vid liječenja ovih tumora je segmentektomija ili još češće ablacija dojke zbog moguće multicentričnosti tumora. Ove tumore još uvijek vidimo veoma rijetko.

Skupina II

T 1a i 1b, No-1a, Mo

Radi se zapravo o bivšem I stadiju gdje se nalazi tumor do 2 cm promjera sa ili bez fiksacije za pektoralnu fasciju ili mišić. Aksila je bez klinički palpabilnih žlijezda, a u slučaju ako se i palpiraju ne smatramo da su zauzete tumorom. Udaljenih metastaza nema.

Kod ovih bolesnica provodi se radikalna mastektomija po Patey-u. Kod tumora lateralnih kvadranta katkad se vrši obična mastektomija s evakuacijom aksile. Tumore samo medijalnih kvadranata dojke dodatno zračimo lokoregionalno telekobaltnom terapijom.

Skupina III

To, 1a, 1b, N1b, Mo
T2a i 2b, No, 1a i 1b, Mo

U ovu skupinu spada primarni tumor već do 5 cm promjera sa ili bez fiksacije za podlogu, te klinički sa ili bez povećanim žlijezdama istostranog pazuha. To je inače klasični II stadij karcinoma dojke.

Kirurški se redovito izvodi modificirana mastektomija po Patey-u u kombinaciji sa standardnom lokoregionalnom radioterapijom. Kod histološki pozitivnih limfnih čvorova bez obzira na broj invadiranih žlijezda primjenjujemo i dodatnu polikemoterapiju, a katkad zavisno o nalazu hormonalnih receptora i hormonalnu terapiju. Sve kirurške zahvate izvodi tim kirurga koji se bavi problematikom dojke.

Postoperativnu telekobaltnu terapiju sprovodimo pomoću tangencijalnih polja za grudnu stijenku i parasternalne žlijezde, dok otvorenu aksilu i supraklavikularno područje zračimo direktnim poljima. Doza je 5000 cGy, frakcionirana tijekom 5 tjedana uz veoma rijetke nuspojave.

Iznimno ako su operacije bile poštedne, onda na uže područje samo primarnog tumora u dojci ili žlijezda u aksili sa manjim poljima dodajemo još 1000—2000 cGy.

Od dodatne kemoterapije potrebljavamo obično 4—6 standardnih kura po CMF shemi sa ili bez Prednisolonom tijekom dvotjedne primjene, rijetko jednokratno. Prema hormonalnoj ovisnosti u skladu sa nalazima receptora do menopauze vršimo ovarijektomiju, a nakon menopauze eventualno primjenjujemo Nolvadex.

DISKUSIJA

Tijekom 20 godišnjeg razdoblja, od 1966—1985 godine, u Rijeci smo liječili ukupno 1.894 bolesnice sa karcinomom dojke različitih stadija. Na temelju stečenog iskustva a i kao mnogi drugi ni mi ne smatramo karcinom dojke sistemnim oboljenjem (Pavlović, Urban 1978). Jer onda lokoregionalna kirurgija i radioterapija ne bi imale smisla, niti bi bile u stanju ni kod ranog stadija izliječiti taj eventualni sistemni tumorski proces.

Uostalom, danas je dobro poznato da od mikroskopske pa do razvoja kliničke faze raka dojke treba da prođe od 2—7 godina. Tako i kod minimalnog karcinoma promjena 0,5 cm do nastanka tumora promjera 1 cm, odnosno ranog kliničkog karcinoma, tumor obično mora proći od 27—30 udvostručenja (Baum 1982). A to su bezbrojne, možda i milionske prilike da neke stanice primarnog tumora diseminiraju po tijelu. Stoga je shvatljivo nastojanje da se rak otkrije upravo dok je još minimalan, odnosno mikroskopske veličine, jer tada još nisu prisutna udaljena metastatska žarišta. U tom slučaju on nije »Sistemno oboljenje« zato što ga sasvim mali kirurški zahvat može potpuno odstraniti, praktički izliječiti u 100% slučajeva (Galager, 1971).

Naravno što je klinički manifestni karcinom veći to je veća i mogućnost limfogenog ili hematogenog rasapa, dok postotak izlječenja proporcionalno pada.

Zato su danas i u svijetu i u nas sva nastojanja usmjerena prvenstveno na ranu detekciju minimalnih tumora ili bar ranog dojke. Broj veoma početnih oblika raka dojke još uvjek je mali da bi rutinski uveli sasvim, male poštedne kirurške zahvate.

Smatramo da su modificirani i reducirani zahvat kod ranih stadija znatno manje mutilirajući i da postižu u kombinaciji sa zračenjem isto tako dobre rezultate kao prijašnja radikalna mastektomija.

Tumorom zauzeta aksila je osnovni i važan indikator širenja primarnog tumora. To je početak diseminacije pa pored zračenja liječenje nadopunjujemo i kemoterapijom, a budućnost će pokazati vrijednost ovog kombiniranog liječenja.

ZAKLJUČAK

Suvremena dijagnostika i terapija ranog raka dojke treba da se sprovodi prema jedinstvenim i usklađenim kriterijima. Zato smo u Kliničkom bolničkom centru Rijeka izradili protokol obrade i liječenja za pojedine stadije raka dojke kojeg se pridržavamo u svakodnevnom radu. Najčešće smo modificirani poštedni kirurški zahvat kombinirali zračenjem a kod tumorom zahvačene aksile i kemoterapijom.

Poštedne zahvate kao što su segmentektomija ili tumerektomija rijetko izvodimo prvenstveno zbog multicentričnog nastanka tumora u cijeloj dojci kao i zbog malog broja veoma početnih tumora. Isto tako smatramo da se karcinom dojke koji se duže razvija samo ponaša kao sistemno oboljenje, dok je početni rak lokoregionalni proces kojeg tako i liječimo. Zato treba otkriti tumor u dojci dok je mali i dok se nije proširio po tijelu.

SAŽETAK

Autori su detaljno iznijeli principe dijagnostičke obrade i liječenja ranog raka dojke koje provode timski u kliničkom bolničkom centru Rijeka.

Radi bolje efikasnosti i jedinstvenog pristupa izradili su detaljno i precizno protokol za pojedine stadije tumora.

Smatrajući rani rak dojke lokoregionalnim procesom a zbog multicentričnog nastanka i tumorom cijele dojke shodno tome su uskladili opsežnost pojedinih terapijskih postupaka.

SUMMARY

PRINCIPES OF TREATMENT OF EARLY BREAST CANCER IN CLINICAL MEDICAL CENTER RIJEKA

The Authors have described the details of diagnostic tests and therapeutic procedures for the early breast carcinoma which are used by the team for breast in the Clinical hospital center of Rijeka.

They have made the precise subjects of protocol for the management of each tumour stage to enable the better eficiency and unanimous approach to the therapy.

The therapeutic procedures are precised according to the Autors opinion that the early carcinoma of the breast is mostly locoregional process and because of its multicentric origin it's also the whole breast tumor.

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SIGNIFICANCE OF SERUM CONCENTRATIONS OF CA 15—3, TPA, CEA, AND FERRITIN IN PATIENTS WITH BREAST CANCER

INTRODUCTION

A circulating tumor marker can be defined as a substance present in a body fluid which qualitatively or quantitatively reflects the presence of malignancy. In certain forms of malignancy, specific biochemical products of tumors, such as human chorionic gonado-tropin (HCG), alpha-feto protein (AFP) and carcinoembryonic antigen (CEA) have proved useful in diagnosing and in monitoring the response to therapy, because their concentrations can be serially measured in the blood (Pluygers 1986, Paulick 1987, Cove 1979). Unfortunately, no such specific marker has yet been described in breast cancer, although a variety of substances have been reported as circulating in abnormally high concentrations in the blood of such patients (Cove 1979, Bezwoda 1981, Lüthgens 1980, Paulick 1987).

The present study was carried out in order to evaluate the clinical significance of 4 different serum tumor markers in a group of subjects with breast cancer. Concentrations of carbohydrate antigen CA 15—3 (CA 15—3), tissue polypeptide antigen (TPA), carcinoembryonic antigen (CEA) and ferritin were determined.

PATIENTS AND METHODS

Seventy-six patients with breast cancer (28 with metastatic disease) were involved in the study. Breast cancer was histologically confirmed in all these patients. The age of the patients ranged from 33—76 years (mean age, 52 years) Metastatic lesions were monitored using X-ray examinations, scintigraphy and, in certain cases, computed tomography. Patients with metastatic disease were treated with local therapy (irradiation) and/or systemic therapy (CMFVP; adriamycin combinations and/or hormonal manipulations). The further course of the disease was monitored by means of standard clinical methods and serial tumor marker analysis at intervals of 2—3 months.

Serum samples were stored at —20 °C prior the assay. CA 15—3 was analyzed by using an immunoradiometric method, IRMA CA 15—3 kit (CIS int.); levels equal to or above 18.9 U/mL were regarded as elevated or positive. TPA was determined by using a RIA kit from Sangtec; titers equal to or above 85 U/L were regarded as positive. CEA was analyzed by using a RIA kit (Mallinckrodt); the normal range was 0—2.5 ng/mL. Ferritin was measured by a radioimmunoassay procedure using a RIA kit from Pharmacia; levels equal to or above 140 μg/L were regarded as elevated or positive.

RESULTS

CA 15—3, TPA, CEA and ferritin values were statistically higher in patients with metastases (Table 1).

The observed positive rates with respect to the clinical course of the disease are given in Table 2.

Table 1.

SERUM CONCENTRATIONS (MEAN ± S.D.) OF CA 15—3, TPA, CEA AND FERRITIN IN PATIENTS WITH BREAST CANCER

	patents with metastatic disease	*patients free of metastases*
CA 15—3 (U/ml)	127,9 ± 19,4	21,1 ± 7,3
TPA (U/L)	354,9 ± 45,3	53,1 ± 32,0
CEA (ng/mL)	48,6 ± 10,1	1,3 ± 1,6
FERRITIN (μg/L)	187,3 ± 15,6	72,4 ± 6,8

Table 2.

SERUM CA 15—3, TPA, CEA AND FERRITIN LEVELS IN BREAST CANCER PATIENTS. RATE OF ELEVATED TITERS IN RELATION TO THE CLINICAL STAGE OF THE DISEASE.

	CA 15—3 20,9 U/mL	*TPA 85 U/L*	*CEA 2,5 ng/mL*	*FERRITIN 140 μg/L*
Patients with metastatic disease	21/28 (75%)	20/28 (71%)	16/28 (57%)	10/28 (35%)
Patients free of metastases	8/48 (17%)	20/48 (41%)	4/48 (8%)	6/48 (12%)

Valeus are number positive per total number.
Values in parentheses are percentages.

Serum CA 15—3 concentrations were raised in 21 patients (75%) with advanced breast cancer. In 17 subjects with CA 15—3 concentrations higher than 50 U/ml and in 4 with values between 23—36 U/ml, metastatic disease was confirmed. CA 15—3 levels were marginaly raised also in 8 patients (17%) with local disease (concentrations between 22—34 U/ml) (Figure 1).

Serum TPA levels were elevated in 20 patients (71%) and CEA in 16 patients (57%) with metastatic disease.

Ferritin concentrations higher than 230 μg/L were invariably associated with metastatic disease.

In three patients serum concentrations of CA 15—3 and CEA were elevated before the start of chemotherapy (CMFVP, cyclophosphamide, methotrexate, 5-fluorouracil, vincristine, prednisone) (Figure 2).

The success of the treatment was monitored by measuring the time course of CA 15—3 and CEA concentrations. There was a dramatic drop in CA 15—3 and CEA concentrations at the start of chemotherapy. On the other hand, in one patient, an increased CEA concentration during chemotherapy was an indication of continued tumor growth.

DISCUSSION

A large number of publications have recently appeared on the use of tumor markers in the diagnosis of malignant tumors, and in monitoring the course of malignancies (Wagener 1984, Pluygers 1986, Paulick 1987).

Among other tumor markers CA 15—3 (Schlom 1984); TPA (Nemoto 1979; Lüthgens 1981; Mross 1985), CEA (Treidler 1984; Neville 1978; Lokich 1987) and ferritin (Jacobs 1976; Bezwoda 1981) have been described as clinical-

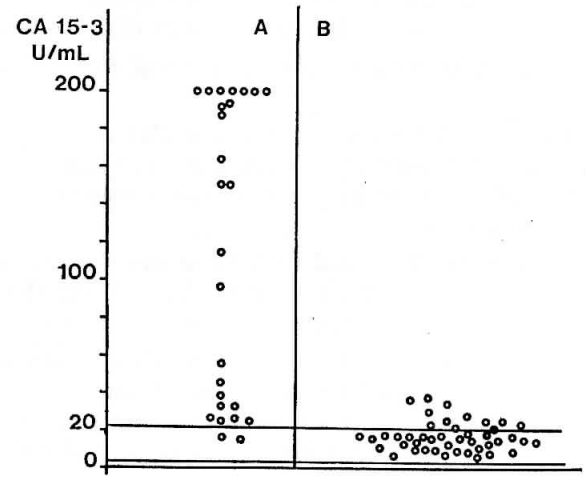

Figure 1. Serum CA 15—3 concentrations in patients with local (B) and advanced (A) breast cancer.

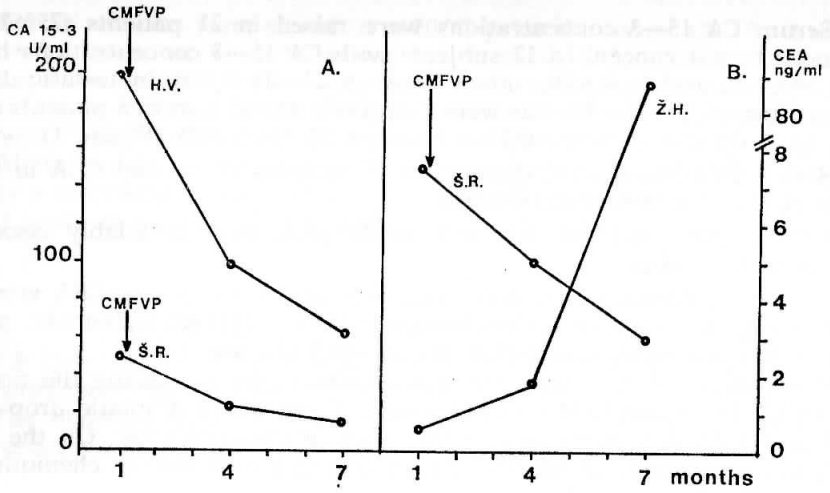

Fig. 2.

Figure 2. Serum CA 15—3 (A) and CEA (B) concentrations before and after progression or regression of advanced breast cancer. CMFVP (cyclophosphamide, methotrexate, 5-fluorouracil, vincristine, prednisone).

ly useful laboratory tools in monitoring patients with breast cancer. Elevated levels of tumor markers ranging from 50—90% were found in metastatic disease (Lokich 1987; Neville 1978; Caffier 1983).

Ca 15—3 is a recently described breast cancer-associated carbohydrate antigen, determined by 2 monoclonal antibodies against human breast cancer (DF3) and milkfat globule (115D8) membranes (Kufe 1984).

Studies comparing the clinical value of several markers for breast cancer are scanty.

Using CEA and phosphohexose isomerase (PHI) in combination, Paulick et al (1987) reported that sensitivity (i.e., at least one marker is elevated) can be increased by 6 to 20%, depending on the clinical stage of the disease, compared to CEA analysis alone.

Serial measurements of TPA and CEA in serum have also been made in order to evaluate their possible usefulness in determining the tumor response to palliative treatment for metastatic disease (Nemoto 1979).

Tormey et al (1977) found CEA useful in predicting the response to treatment and the prognosis in patients with breast cancer and there are studies in which ferritin concentrations have been shown to correlate with the extent of the disease on patients with breast cancer (Jacobs 1976, Marcus 1975, Torizuka 1979).

This study indicates that marker analysis in breast cancer may be used in the follow-up of patients for detection of metastases, in monitoring effectiveness of treatment, and in palliative therapy of advanced disease. The

comparison of tumor markers showed that CA 15—3 has the greatest sensitivity for breast cancer, followed by TPA and CEA. Ferritin was a less sensitive discriminator.

Raised ferritin concentrations appear to reflect hepatic metastases; the results of this study indicate that they were raised in patients exhibiting such metastases. The reason for this is perhaps the release of normal ferritin from hepatic cells damaged by the tumor infiltrate, because they were invariably present in patients with serum concentrations higher than 230 μg/L.

In addition, a combination of antigens provides greater sensitivity than a single marker.

There are different ways to combine two or more tests. One way is to form a product value of the two markers (CEA × TPA, CEA × CA 15—3, CA 15—3 × TPA).

If they are used in appropriate combinations with other diagnostic procedures, and the time course of marker concentration is taken into account, the tumor markers can extend the possibilities for tumor diagnosis.

Tumor markers are particulary effective in the early recognition of local relapses and metastases, and for the sensitive and objective monitoring of chemotherapy.

We conclude that three markers tested (CA 15—3, TPA, CEA) are clinically useful laboratory tools in monitoring patients with breast cancer. Positive rates and mean values are associated with extended tumor burden and tumor activity. The use of multiple tumor marker analysis results in an increased sensitivity without loss of specificity and should be preferred to single marker analysis.

SUMMARY

Serum concentrations of carbohydrate antigen 15—3 (CA 15—3), tissue polypeptide antigen (TPA), carcinoembryonic antigen (CEA) and ferritin were measured in 76 patients with breast cancer, 28 of whom had metastatic disease. CA 15—3, TPA, CEA and ferritin values were statistically higher in patients with metastases. Serum CA 15—3 concentration was raised in 21 (75%) patients with advanced breast cancer. Serum TPA level was raised in 20 patients (71%) and CEA level in 16 patients (57%) with metastatic disease. Ferritin concentration was raised in 5 patients (18%) all with hepatic metastases. When two or three tumor markers were combined, the diagnostic accuracy increased.

SAŽETAK

ZNAČENJE SERUMSKIH KONCENTRACIJA CA 15—3, TPA, CEA I FERRITINA U BOLESNICA SA RAKOM DOJKE

U serumu 76 bolesnica s karcinomom dojke određena je koncentracija ugljikohidratnog antigena CA 15—3 (engl. carbohydrate antigen CA 15—3), tkivnog polipeptidnog antigena TPA (engl. tissue polypeptide antigen), karcinoembrionskog antigena CEA (engl. carcinoembryonic antigen) i feritina. Metastaze su dokazane u 28 bolesnica. Razina svih tumorskih obilježivača bila je statistički značajno povišena u bolesnica s metastazama. U serumu 21 (75%) bolesnice s uznapredovalim karcinomom dojke utvrđena je povišena koncentracija CA 15—3 u serumu. Povišena koncentracija TPA u serumu utvrđena je u 20 (71%) bolesnica, a CEA u 16 (57%) bolesnica s metastazama. Razina feritina bila je povišena u serumu 5 (18%) bolesnica s metastazama u jetri. Istovremenim određivanjem dva ili više tumorskih obilježivača i uspoređivanjem njihovih vrijednosti povećana je mogućnost dijagnoze metastaza.

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SUBTUMORAL AND INTRATUMORAL APPLICATION OF HUMAN NATURAL LEUCOCYTIC INTERFERON AND RECOMBINANT HUIFN ALPHA 2C IN PATIENTS WITH CANCERS OF THE HEAD AND NECK

Ca. basocellulare (Basal cell carcinoma) is infiltratable locally. As a rule, it does not lead to metastasis. It is generally localized in the upper third of the face, and is made up of basal cells of the epidermis. The prognosis is favorable with modern surgical therapy. It is not favorable only in extremely advanced casese which may lead to destruction of tissue all the way to the bone, and to deformation and mutilation of often considerable size.

Ca. planocellulare (Squamous cell carcinoma) is an epidermal invasive cancer of the skin and visible mucous membranes made up of spinous epidermal cells. A high percentage of undifferentiated cells without keratotic pearls indicates malignancy of the tumor. Squamous cell carcinoma of the lower lip larger than 2 cm. metastasizes in 25% or more cases, and on the tongue in 80% of cases.

Our first results with local application of HNLI in patients with ca. basocellulare of the skin were published in 1975 (Ikić et al. 1975). Results from the following 17 of our patients (ca. basocellulare and ca. planocellulare) were treated with human natural leucocytic interferon were published in Lancet in 1981 (Ikić et al. 1981 a.). On the basis of second pathohistological biopsy taken immediately following the end of therapy from the site where the carcinoma had been located, results were negative in 14 of the 17 cases. In the research we are now carring out, human natural leucocytic interferon (HNLI) and recombinant HuIFN alpha 2c are used in the therapy of ca. basocellulare and ca. planocellulare.

PATIENTS AND METHODS

Patients:

Twenty-three patients with ca. basocellulare and ca. planocellulare were treated with HNLI (Table 1). Fifteen patients were male and eight female,

aged 41—81 years. The sites of the tumors were: lower lip — 2, forehead — 4, tonsils — 2, nose — 7, ear shell — 4, face — 4. The pathohistological diagnosis made prior to the application of natural leucocytic interferon was: 15 cases with ca. basocellulare and 8 cases with ca. planocellulare. Tumor size before treatment: longer diameter, 10—20 mm. and shorter diameter, 5—15 mm.

Eighteen patients with ca. basocellulare and ca. planocellulare were treated with recombinant HuIFN alpha 2c (Table 2). Ten patients were male and eight female, aged 49—86 years. The sites of the tumors were: lower lip — 4, forehead — 5, nose — 4, ear shell — 1, face — 2, retroauricular — 1, sulcus nasolabiale — 1. The pathohistological diagnosis prior to treatment with recombinant HuIFN alpha 2c was: 10 cases with ca. basocellulare and 8 cases with ca. planocellulare. Tumor size before treatment: longer diameter, 5—20 mm. and shorter diameter, 2—15 mm.

Interferon:

The duration of treatment with semipurified concentrated HNLI (10^4 units per 1mg. protein) was 3—6 weeks. A simple dose was 400.000—1.200.000 units. The number of application ranged from 12—30 times, and the total dose was between 5,6—21,6 milion units. The interferon was administered in 1 cc. with an intradermal needle (one injection but changing direction), causing both subtumoral and intratumoral infiltration. No other medication was administered during the course of the interferon treatment. The HNLI was produced at the Institute of Immunology in Zagreb, Yugoslavia (Table 4).

The duration of treatment with recombinant HuIFN alpha 2c was 4 weeks. Fifteen patients received 20 subtumoral and intratumoral injections of 2.000.000 units of r.IFN alpha 2c (total dose: 40 million), and three patients (nos. 16, 17 and 18) received 20 subtumoral and intratumoral injections of 5.000.000 units (total dose: 100 million units). The recombinant IFN alpha 2c was applied daily except for Saturday and Sunday. No other medication was administered during treatment. The recombinant IFN alpha 2c was produced by Boehringer Ingelheim and was donated to us free of charge (Table 3).

Pathohistological evaluation of results:

Biopsy material was taken from the edge of the tumor, and from deep enough to include all layers affected by tumorous growth. Material was taken during the first examination and immediately following treatment, within 72 hours. In cases where the tumor had disappeared, the biopsy material was taken from the site where the tumor had been. In cases where the tumor persisted after treatment with interferon, the tumor was surgically removed, in which case the tumor represented the material for biopsy. The biopsy material was conserved in 10% formalin, placed in paraffin and was prepared with a Reichert microtome (10 microns) and dyed with hematoxilin-eosin.

Clinical evaluation of results:

0 = no effect
\+ = poor effect — tumor shows tendency towards regression (25%)

++ = satisfactory effect — size of tumor has significantly regressed (25—75%), clearly circumscribed
+++ = very good effect — size of tumor regressed more than 75%, or is present as barely visible nonepithelialized lesion
++++ = excellent effect — tumor has disappeared, fully epithelialized

Immunohistological evaluation of results:

0 = no response, non-reactive stroma
\+ = poor response, scattered lymphoid cells
++ = moderate response, numerous lymphoid cells, narrow infiltration areas, few macrophages
+++ = good response, massive infiltration of lymphoid cells, numerous macrophages
++++ = very good response, massive infiltration of lymphoid cells, numerous macrophages, tumorous cells have disappeared

RESULTS

Results of the recombinant HuIFN alpha 2c therapy are summarized in Table 3. According to pathohistological and clinical findings, nine out of 18 patients (nos. 1, 4, 7, 8, 9, 10, 12, 16 and 17) were cured. Four of the eight patients with squamous cell carcinomas (nos. 4, 12, 16 and 17) were considered cured according to pathohistological findings. Five of the ten patients with basal cell carcinomas (nos. 1, 7, 8, 9 and 10) were considered cured. One of the four patients with squamous cell carcinoma on the lower lip (no. 17) was considered cured. In two patients with squamous cell carcinoma (nos. 3 and 15), there was a 25% reduction in tumor size, and in one patient (no. 6), there was a more than 75% reduction in tumor size: in all three cases, the carcinoma was removed surgically with no disfigurement. In one patient (no. 18), clinical findings showed the tumor to have disappeared, but tumor cells were still found in the material taken after interferon treatment for the second biopsy. This patient would probably have had negative pathohistological results had the therapy lasted four weeks longer. Tumor size was reduced more than 75% in two patients (nos. 6 and 13), about 50% in patients (nos. 2, 5, 14), and about 25% in patients (no. 3, 15) and not reduced in one patient (no. 11). It can be said that the interferon treatment was succesful in 17 of the 18 patients.

Results of the human natural leucocytic interferon are summarized in Table 4. Both clinically and histologically, 18 of the 23 patients (nos. 2, 3, 6, 7, 8, 9, 10, 11, 13, 14, 16, 17, 18, 19, 20, 21, 22 and 23) were considered cured. The tumor size was reduced about 50% in two patients (nos. 1 and 4), about 25% in two patients (nos 12 and 15), and in one patient (no. 5) was not reduced at all. Eleven of the 14 patient with basal cell carcinoma (nos. 2, 8, 9, 10, 13, 16, 17, 20, 21, 22 and 23) were considered cured. Seven of the 9 patients with squamous cell carcinoma (nos. 3, 6, 7, 11, 14, 18 and 19) were considered cured. Lower lip tumors, if extensive, leave the patient disfigured after surgery. One of the two patients with carcinoma on the lower lip (no. 3) was

considered cured after treatment and no surgery was necessary. In the second case, the tumor was reduced by 50% in size and was surgically removed without disfigurement. In one patient (no. 5), the tumor was not clinically diminished after the interferon treatment was completed.

Case reports (r.HuIFN alpha 2c)

Patient: 1

Clinical finding before treatment:
Tumor lies in middle of forehead (10 × 5 mm.), is dark brown in color, elevated from the skin surface, and has a small ulcer in the center.

Pathohistological finding before treatment:
Tumorous formation in corium, composed of multicentric solid foci, atypical epithelial cells of oval and elongated shape which are cylindrical peripherally and of palisade arrangement.
DG: Carcinoma basocellulare.

Clinical finding after treatment:
There is a large scar, skin is reddish on the site of interferon application. Clinically, tumor has disappeared.

Pathohistological finding after treatment:
Cancer cells have disappeared. Proliferation and infiltration of lymphoid cells.
DG: Sine carcinomate. Cicatrix.

Patient: 2

Clinical finding before treatment:
Tumor is located on the forehead (10 × 20 mm.).

Pathohistological finding before treatment:
Tumorous tissue in corium, composed of smaller foci of spindle shaped hyperchromatic cells of cylindrical arrangement.
DG: Carcinoma basocellulare

Clinical finding after treatment:
Lesion has diminished to one third original size. Marked epithelialization from periphery to center. Tumor presence visible in center. Edges of lesion sharply defined.

Pathohistological finding after treatment:
Tumor is composed of multiple smaller foci, atypical epithelial cells of palisade arrangement peripherally.
DG: Carcinoma basocellulare

Patient: 3

Clinical finding before treatment:
Tumor is on the lower lip (15 mm. diam.). Small ulcer in the center.

Pathohistological finding before treatment:
Tumor is largely composed of the focus of a ray of atypical columnar epithelial cells. Necrosis with minor ulceration observable at one spot. Abundant lymphoid infiltration of stroma with numerous accumulations of lymphocytes and plasma cells.
DG: Carcinoma planocellulare

Clinical finding after treatment:
Tumor has diminished approx. 25% in size. Pronounced inflammatory reaction surrounding the tumor.

Pathohistological finding after treatment:
Pronounced granulomatous reaction around the elements of carcinoma planocellulare. Cancer of mature type without mitoses. Lymphocytes and plasmocytes prevalent in inflammatory stroma, macrophages scarce.
DG: Carcinoma planocellulare

Patient: 4

Clinical finding before treatment:
Tumor is on the forehead (5 × 10 mm.), is somewhat ulcerated and at skin level.

Pathohistological finding before treatment:
Small foci of atypical columnar epithelial cells surrounded by pronounced infiltration of lymphocytes and plasma cells.
DG: Carcinoma planocellulare microinvasivum

Clinical finding after treatment:
Lesion has fully epithelialized.

Pathohistological finding after treatment:
Cancer cells have disappeared. Scarred tissue in corium with small foci of lymphocytes and plasma cells.
DG: Sine tumore

Patient: 5

Clinical finding before treatment:
Tumor is on the forehead (20 × 10 mm.), above skin level, exulcerated, brown pigmentated, bleeds profusely upon pressure.

Pathohistological finding before treatment:
Tumor formed in corium of solid foci of small atypical epithelial cells of palisade arrangement peripherally.
DG: Carcinoma basocellulare

Clinical finding after treatment:
Tumor diminished to one half original size. Exulcerated edges fully epithelialized. Pronounced surrounding inflammatory reaction.

Pathohistological finding after treatment:
Tumor composed of solid masses of small atypical epithelial cells of palisade arrangement peripherally. Mitoses in most tumor cells. Pronounced infiltration of

lymphoid and plasma cells around the tumor.
DG: Carcinoma basocellulare

Patient: 6

Clinical finding before treatment:
Tumor is on the lower lip (10 × 5 mm.), uneven edges, brown color, bleeds profusely to the touch.

Pathohistological finding before treatment:
Tumor is composed of solid accumulations and rays of atypical columnar cells which form balls of horny matter.
DG: Carcinoma planocellulare

Clinical finding after treatment:
Remainder of tumor (3 × 1 mm.) is clearly defined, somewhat above skin level and does not bleed to the touch. Local reaction is quite pronounced.

Pathohistological finding after treatment:
Tumor is composed of accumulations and rays of atypical columnar epithelial cells which sporadically form balls of horny matter. Tissue below the ulcer and surrounding the tumor contains abundant dense accumulations of lymphoid cells.
DG: Carcinoma planocellulare

Patient: 7

Clinical finding before treatment:
Tumor lies in the nasolabial sulcus (10 × 10 mm.), is above skin level, dark brown pigmented, exulcerated and bleeds to the touch.

Pathohistological finding before treatment:
Tumor in corium, composed of foci of atypical oval and cylindrical eipthelial cells of palisade arrangement peripherally.
DG: Carcinoma basocellulare

Clinical finding after treatment:
Tumor has completely epithelialized. Inflammatory reaction around the tumor is very pronounced.

Pathohistological finding after treatment:
Cancer cells have disappeared. Epidermis is swollen and shows disordered stratification as well as atypia of cells. Dense accumulations of lymphoid and plasma cells found in the corium around sebaceous glands and hair follicles, and sporadically perivasularly and in the solid tissue.
DG: Sine tumore

Patient: 8

Clinical finding before treatment:
Tumor is on the forehead (10 × 10 mm.), is exulcerated, above skin level, dark brown pigmented, with uneven edges and bleeds to the touch.

Pathohistological finding before treatment:
Tumor composed of cells resembling basal layer epidermal cells, of palisade arrangement.
DG: Carcinoma basocellulare

Clinical finding after treatment:
Tumor site is sharply defined, fully epithelialized and on the skin level. Local reaction pronounced.

Pathohistological finding after treatment:
Atrophic epidermis from compression of collagen tissue which grows into corium. Folliculoid accumulations of lymphoid cells in tissue. No cancer cells.
DG: Sine carcinomate

Patient: 9

Clinical finding before treatment:
Tumor is on face below the hairline (10 × 20 mm.), ulcer with uneven edges brown pigmentation.

Pathohistological finding before treatment:
Solid foci of small atypical epithelial cels in corium, of palisade arrangement peripherally.
DG: Carcinoma basocellulare

Clinical finding after treatment:
Tumor has been cicatrized.

Pathohistological finding after treatment:
No tumor cells. Zone of multiple fibrous connections on tumor site. Adjacent skin hypertrophic. Accumulations of lymphoid cells disseminated around site. Epidermis normal, very atrophied. Inflammatory infiltration along the wide venules as well.
DG: Sine tumore

Patient: 10

Clinical finding before treatment:
Tumor is on tip of nose (5 × 5 mm.), brown pigmentation with small ulcer and uneven edges.

Pathohistological finding before treatment:
Tumor in upper corium composed of foci of atypical epithelial cells resembling basal level epidermal cells, of palisade arrangement on periphery. Profuse lymphoid infiltration around tumor.
DG: Carcinoma basocellulare

Clinical finding after treatment:
Tumor has completely disappeared. Defect epithelialized.

Pathohistological finding after treatment:
No tumor cells found. Profuse infiltration of lymphoid cells and microphages in corium and scar tissue.
DG: Sine tumore

Patient: 11

Clinical finding before treatment:
Tumor is on the face above the hair line (20 × 20 mm.), reddish brown pigmentation, heavily vascularized, slightly inflamed on the edges. Exulceration in the center of the tumor, covered with a crust. Profuse bleeding to the touch. Tumor edges not sharply defined.

Pathohistological finding before treatment:
Tumor is composed of foci and rays of atypical flat epithelial cells, in some places forming small balls of horny matter.
DG: Carcinoma planocellulare

Clinical finding after treatment:
Size of tumor unchanged.

Pathohistological finding after treatment:
Tumor composed of foci and rays of atypical flat epithelial cells which form small balls of horny matter. Tumor infiltrates the depth of the corium and penetrates the subcutaneous fat tissue. Profuse infiltration of lymphoid cells around the tumor. Tumor zone surrounded by cellular connective tissue full of lymphoid cells.
DG: Carcinoma planocellulare

Patient: 12

Clinical finding before treatment:
Tumor is on the left auricle (20 × 20 mm.), deep ulcer, appears to have caught the cartilage.

Pathohistological finding before treatment:
Tumor is composed of foci of atypical flat epithelial cells. Stroma around tumor is profusely infiltrated with lymphoid cells which in places are folliculoid.
DG: Carcinoma planocellulare

Clinical finding after treatment:
Tumor has completely disappeared.

Pathohistological finding after treatment:
Wide blood spaces with thick walls, rays of connective tissue. Carcinoma cells have disappeared.
DG: Sine carcinomate

Patient: 13

Clinical finding before treatment:
Tumor is on the nose (10 × 20 mm.), covering nearly the entire nasal wing, is exulcerated, above hair level, with uneven edges.

Pathohistological findings before treatment.
Tumor composed of foci of small atypical epithelial cells in the corium, of palisade arrangement on the periphery. In between the foci of tumor cells in places are more abundant connective stroma with sparse accumulations of lymphocytes
DG: Carcinoma basocellulare

Clinical finding after treatment:
Tumor size reduced 2 × 2 mm. Pronounced cicatricion and epithelialization of the tumor from the periphery to the center. No signs of exulceration, crust in the center of the lesion.

Pathohistological finding after treatment:
Tumor composed of small solid masses and narrow rays of small atypical epithelial cells of palisade arrangement peripherally. Between the rays of tumor cells, abundant hard collagen stroma are found, as well as in places dense infiltration of lymphoid cells. Proliferation of fibrous connective tissue full of lymphoid cells around the rays. Lymphoid cells are massed around hypertrophic full sebaceous glands.
DG: Carcinoma basocellulare

Patient: 14

Clinical finding before treatment:
Tumor is on the nose (5 × 10 mm.), ulcerous.

Pathohistological finding before treatment:
Tumor composed of small atypical epithelial cells of palisade arrangement peripherally. Profuse infiltration of lymphocytes and plasma cells with some macrophages around the tumor site.
DG: Carcinoma basocellulare

Clinical finding after treatment:
Remains of tumor at skin level, sharply defined, with no signs of exulceration. Two thirds of original tumor completely epithelialized from the edges to the center.

Pathohistological finding after treatment:
Epidermis atrophied, corium contains hyaline connective tissue. Small flat epithelial cells, branching and forming interconnecting rays. Tumor stroma full of lymphoid cells.
DG: Carcinoma planocellulare

Patient: 15

Clinical finding before treatment:
Tumor is on the mucous membrane of the lower lip (15 × 5 mm.), brownish red pigmentation, below the membrane level.

Pathohistological finding before treatment:
Tumor composed of atypical flat epithelial cells which form rays and foci to 2.5 mm. in depth. Profuse lymphoid infiltration around tumor. Tumor penetrates to the lateral edge of the exision.
DG: Carcinoma planocellulare

Clinical finding after treatment:
Tumor has diminished 25% and become defined from the surroundings.

Pathohistological finding after treatment:
Tumor composed of accumulations and foci of atypical flat epithelial cells. Tumor shallowly infiltrates the transversal striate muscle, and is surrounded by dense

infiltration of lymphoid cells. Dense perivascular infiltration of lymphocytes and macrophages found deep in the muscle and connective tissue, and in places dense infiltration of the blood vessel walls.
DG: Carcinoma planocellulare

Patient: 16

Clinical finding before treatment:
The tumor is a recrudescent tumor (5 × 5 mm.) on the root of the nose after surgery, above skin level, brown pigmentation with uneven edges.

Pathohistological finding before treatment:
Tumor tissue composed of accumulations of atypical flat epithelial cells with horny matter in their centers. Lymphoid infiltration around the tumor accumulations.
DG: Carcinoma planocellulare

Clinical finding after treatment:
Tumor has diminished and epithelialized.

Pathohistological finding after treatment:
Numerous bundles of fibroblasts and fibrocytes in the corium, with some capillaries and broad rays of collagen with hyalin fibers; surrounded by profuse perivascular infiltration by lymphocytes and plasma cells. No tumor cells found.
DG: Cicatrix. Sine tumore

Patient: 17

Clinical finding before treatment:
Tumor is on the lower lip (5 × 2 mm.).

Pathohistological finding before treatment:
Tumor cells in some places spindle shaped with hyperchromatic basophilic nuclei, penetrating in foci and rays to 1 mm. in depth. Marked profuse inflammatory infiltration by lymphocytes, plasma cells, eosinophils and macrophages.
DG: Carcinoma planocellulare

Clinical finding after treatment:
Tumor disappeared completely.

Pathohistological finding after treatment:
No tumor cells found. Marked infiltration of lymphoid cells in the stroma.
DG: Sine tumore

Patient: 18

Clinical finding before treatment:
Tumor lies in the left auricle (5 × 5 mm.), brown pigmentation, beginnings of an ulcer, with uneven edges.

Pathohistological finding before treatment:
Tumor composed of solid masses atypical oval epithelial cells of uniform appearance with dark grey nuclei and scanty cytoplasm, of palisade arrangement peripherally.
DG: Carcinoma basocellulare

Clinical finding after treatment:
Macroscopically tumor has disappeared completely.

Pathohistological finding after treatment:
Solid foci of small atypical epithelial cells of palisade arrangement peripherally. In the stroma, profuse infiltration by lymphoid cells.
DG: Carcinoma basocellulare

DISCUSSION

We would not expect r.IFN alpha 2c to be more efficacious than HNLI. The clinical efficacy of r.IFN alpha 2c can only approximate the clinical efficacy of HNLI, which is shown in this research. However, the advantage of r.IFN alpha 2c lies in the fact that it can be produced in practically unlimited quantities, where as the production of HNLI is dependent upon a limited amount of human leucocytes. Both types of interferon, r.IFN alpha 2c and HNLI, are both clinically and pathohistologically extremely effective. They do not differ significantly, but is seems that HNLI is more effective, because doses of lower concentration are necessary, and the rate of cure is higher.

There is no significant difference in the clinical degree of cure with regard to the diagnosis of basal cell carcinoma or squamous cell carcinoma. Histologically, HNLI and r.IFN alpha 2c do not induce any specific response that is not present in the normal interaction between tumor cells and the immune system. There is no difference between HNLI and r.IFN alpha 2c in the immunological response. Prior to therapy with interferon, as a rule there are few cellular elements in the tumor stroma. After interferon application, the stroma, as a rule, is widely filled with masses of lymphocytes and macrophages which enter the rays of the tumor cells and destroy them. Occasionally, an accumulation of lymphocytes similar to lymph nodes are formed at the tumor site. Connective tissue grows at the tumor site. The histological findings are the same in cases of carcinomas of the head and neck, and in cases of carcinomas of the breast and cervix (Ikić et al. 1981 a; Ikić et al. 1981 b; Ikić et al. 1981 c; Knežević et al. 1982, Padovan et al. 1981.).

Interferon acts upon the immune system and upon the cancerous cell (Rubinstein and Orchansky, 1986; Sreevalsan et al. 1986; De Maeyer-Guignard and De Maeyer, 1986.). The action of interferon on the cancer cell is essential. The effect of this action is that the cancer cells multiply more slowly and become less invasive, which makes it possible for the immune system to act more successfully, eventually leading to necrosis of the cancer cells (Ikić et al. 1976 Belardelli et al. 1983.). The degree of infiltration of the tumor stroma by lymphoid cells depends upon the invasiveness of the tumor. The less invasive the cancer cells, the stronger the infiltration of the stroma by lymphoid cells, and vice versa — the more invasive the cancer cells, the less infiltration of the stroma by lymphoid cells. The anti-invasive action of inter-

feron, which can be measured (Ikić et al. 1982). allows a stronger infiltration of the stroma by lymphoid cells, and the presence of interferon certainly has a positive effect on their action. The direct influence of interferon on cancer cells can be seen in tissue culture. If we add LED-Widr cancerous cells to human diploid (HD) cells grown in a confluent layer, after three days fissures will be visible in the HD cell layer. Cancer cells will be found in most of these fissures, either alone or in colonies. Cancerous LED-Widr cells have a polygonal shape with a large nucleus. If interferon is added to the HD cells along with LED-Widr cancerous cells, the HD cells will not be broken up by fissures and there will be fewer cancer cells. The cancer cells themselves will be round and shrivelled, have much less protoplasm with indefinite borders, and the nuclei appear to be amalgamated with the scenty protoplasm into one unit.

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Table 1

HNLI. TUMOURS OF THE HEAD AND NECK

Patient No.	*Age*	*Sex*	*Tumor site*	*Pretreatment size in mm*
1	78	M	Lower lip	20 × 15
2	52	M	Forehead	20 × 10
3	58	M	Lower lip	15 × 15
4	50	M	Left tonsil	10 × 10
5	48	M	Face infraorbitally	20 × 10
6	41	F	Tonsils	15 × 10
7	60	F	Forehead	10 × 10
8	42	F	Forehead	10 × 10
9	38	M	Nose	20 × 10
10	81	M	Ear	20 × 20
11	42	M	Ear	10 × 5
12	68	F	Face	10 × 10
13	79	F	Face	20 × 15
14	43	M	Ear	15 × 15
15	72	M	Ear	15 × 15
16	55	M	Nose	15 × 15
17	52	M	Nose	10 × 10
18	48	M	Nose	15 × 10
19	55	M	Nose	10 × 10
20	72	F	Nose	15 × 10
21	74	F	Nose	15 × 15
22	48	F	Forehead	10 × 10
23	64	M	Face	10 × 5

Table 2

R.IFN alpha 2c TUMOURS OF THE HEAD AND NECK

Patient No.	*Age*	*Sex*	*Tumor site*	*Pretreatment size in mm*
1	86	M	Skin of forehead	10 × 5
2	53	F	Skin of forehead	20 × 10
3	55	M	Lower lip	15 × 15

4	50	F	Skin of forehead	10 × 5
5	49	M	Skin of forehead	20 × 10
6	56	M	Lower lip	10 × 5
7	62	F	Sulcus nasolabiale	10 × 10
8	61	F	Skin of forehead	10 × 10
9	63	M	Skin of face	20 × 10
10	66	F	Skin of nose	5 × 5
11	68	F	Skin of face	20 × 5
12	53	M	Skin of ear	20 × 20
13	70	F	Skin of nose	20 × 10
14	68	M	Skin of nose	10 × 5
15	81	F	Lower lip	5 × 5
16	66	M	Root of nose	5 × 5
17	64	M	Lower lip	5 × 2
18	73	M	Retroauricular left	5 × 5

Table 3

R.IFN alpha 2c TUMOURS OF THE HEAD AND NECK

Patient No.	Pathohistological findings		Duration of treat. (weeks)	No. of applic.	Total units (x 10⁶)	Immunohistol. grade		
	before treatment	after treatment				before treatment	after treatment	Clinic. grade
1	Ca. basocellulare	Sine tm.	4	20	40	0	++++	++++
2	Ca. basocellulare	Ca. basocellulare	4	20	40	++	++	++
3	Ca. planocellulare	Ca. planocellulare	4	20	40	+++	+++	+
4	Ca. planocellulare	Sine tm.	4	20	40	+++	++++	++++
5	Ca. basocellulare	Ca. basocelluulare	4	20	40	++	+++	++
6	Ca. planocellulare	Ca. planocellulare	4	20	40	+	+++	+++
7	Ca. basocellulare	Sine tm.	4	20	40	+++	++++	++++
8	Ca. basocellulare	Sine tm.	4	20	40	0	++++	++++
9	Ca. basocellulare	Sine tm.	4	20	40	+++	++++	++++
10	Ca. basocellulare	Sine tm.	4	20	40	+++	++++	++++
11	Ca. planocellulare	Ca. planocellulare	4	20	40	++	+++	0
12	Ca. planocellulare	Sine tm.	4	20	40	+++	++++	++++
13	Ca. basocellulare	Ca. basocellulare	4	20	40	++	+++	+++
14	Ca. basocellulare*	Ca. planocellulare	4	20	40	+++	+++	++
15	Ca. palnocellulare	Ca. planocellulare	4	20	40	+++	+++	+
16	Ca. planocellulare	Sine tm.	4	20	100	+++	++++	++++
17	Ca. planocellulare	Sine tm.	4	20	100	+++	++++	++++
18	Ca. basocellulare	Ca. basocellulare	4	20	100	+++	+++	++++

* probably Ca. basocellulare mix tum.

Table 4

HNLI. TUMOURS OF THE HEAD AND NECK

Patient No.	Pathohistological findings		Duration of treat. (weeks)	No. of applic.	Single dose (x 10⁶)	Immunohistol grade after treatment	Clinical grade
	before treatment	after treatment					
1	Ca. planocellulare	Ca. planocellulare	5	24	0.8	++	++
2	Ca. basocellulare	Sine tm.	3	15	1.2	++++	++++
3	Ca. planocellulare	Sine tm.	4	18	1.2	++++	++++
4	Ca. planocellulare	Ca. planocellulare	5	24	0.5	+++	++
5	Ca. basocellulare	Ca. basocellulare	6	30	0.4	0	0
6	Ca. planocellulare	Sine tm.	3	14	0.4	++++	++++
7	Ca. planocellulare	Sine tm.	3	12	0.8	++++	++++
8	Ca. basocellulare	Sine tm.	5	25	0.4	++++	++++
9	Ca. basocellulare	Sine tm.	5	24	0.4	++++	++++
10	Ca. basocellulare	Sine tm.	4	15	0.4	++++	++++
11	Ca. planocellulare	Sine tm.	5	18	0.4	++++	++++
12	Ca. basocellulare	Ca. basocellulare	6	30	0.4	+	+
13	Ca. basocellulare	Sine tm.	5	20	0.8	++++	++++
14	Ca. planocellulare	Sine tm.	5	15	0.4	++++	++++
15	Ca. basocellulare	Ca. basocellulare	4	20	0.4	+	+
16	Ca. basocellulare	Sine tm.	6	30	0.5	++++	++++
17	Ca. basocellulare	Sine tm.	6	30	0.5	++++	++++
18	Ca. planocellulare	Sine tm.	6	30	0.5	++++	++++

19	Ca. planocellulare	Sine tm.	6	30	0.5	++++	++++
20	Ca. basocellulare	Sine tm.	6	30	0.5	++++	++++
21	Ca. basocellulare	Sine tm.	6	30	0.5	++++	++++
22	Ca. basocellulare	Sine tm.	6	30	0.5	++++	++++
23	Ca. basocellulare	Sine tm.	6	30	0.5	++++	++++

SUMMARY

Totally 23 patients have been treated with HNLI (ca. basocellulare 14 cases and ca. planocellulare 9 cases) and 18 patients with r.IFN alpha 2c (ca. basocellulare 10 cases and ca. planocellulare 8 cases). Duration of treatment was 3—6 weeks and 400.000 — 1.200.000 units of HNLI were applied daily except for Saturday and Sunday. Duration of treatment in case of r.IFN alpha 2c was 4 weeks, single dose was 4 weeks, single dose was 2.000.000——5.000.000 units applied daily except for Saturday and Sunday. Bioptic material was taken before and after the treatment. After the treatment with HNLI in 17 out of 23 patients cancerous cells were not found in the material taken after interferon treatment for the second biopsy. After the treatment with r.IFN alpha 2c in 9 patients out of 18 cancerous cells were not found in the second biopsy. Immunohistological process of cure of tumours with HNLI and r.IFN alpha 2c has been the same. Both types of interferon r.IFN alpha 2c and HNLI are both clinically and pathohistologically very effective.

SAŽETAK

SUBTUMORSKA I INTRATUMORSKA APLIKACIJA LJUDSKOG NARAVNOG LEUKOCITARNOG INTERFERONA I REKOMBINANTA HuIFN ALPHA 2c KOD BOLESNIKA SA RAKOM GLAVE I VRATA

Osamnaest bolesnika oboljelih od ca. basocellulare i ca. planocellulare liječeni su rekombinantnim HuIFN alpha 2c. Rezultati su prikazani u tablici 3. Prema patohistološkom i kliničkom nalazu izliječeno je 9 od 18 bolesnika (u tablici 3 broj: 1, 4, 7, 8, 9, 10, 12, 16, 17). U drugoj biopsiji naprijed navedenih bolesnika nisu nađene tumorske stanice. Kod ostalih je bolesnika smanjena veličina tumora (u tablici 3 broj: 2, 3, 5, 6, 13, 14, 15, 18).

Dvadeset tri bolesnika oboljela od ca. basocellulare i ca. planocellulare liječeni su humanim prirodnim leukocitnim interferonom (HNLI). Rezultati su prikazani u tablici 4. Prema patohistološkom i kliničkom nalazu izliječeno je 18 od 23 pacijenta (u tablici 4 broj: 2, 3, 6, 7, 8, 9, 10, 11, 13, 14, 16, 17, 18, 19, 20, 21, 22, 23). Kod ostalih bolesnika (broj: 1, 4, 12, i 15) smanjena je veličina tumora. Kod jednog bolesnika (broj 5) poslije završene terapije veličina tumora nije bila smanjena. Terapija s HNLI i ca. basocellulare i ca. planocellulare bila je vrlo uspješna. Za vrijeme terapije rekombinantnim HuIFN alpha 2c i prirodnim humanim leukocitnim interferonom pacijenti nisu primali nikakvu drugu terapiju.

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APPLICATION OF INTERFERON IN PATIENTS WITH CARCINOMA BASOCELLULARE

Interferon can be considered an important therapy for many dermatological conditions resistant to standard treatment. Our first results with local application of human natural leucocytic interferon (HNLI) in dermatological diseases were published in the early 1970 s. (Ikić, D., et al. 1971, 1972, 1973, 1974, 1975 a, b, c, 1981.).

The therapeutic effect of HNLI on benign tumor condylomata acuminata was evaluated in a double-blind placebo-controlled clinical trial. The therapeutic effect of HNLI was statistically significant (Ikić, D., et al. 1975 a, b, c, 1981).

Genital warts or condylomata acuminata were treated succesfully by different investigators as well (Scott, G. M., et al. 1979; Eron, L;. J., et al. 1986; Vance, J. C., et al. 1986).

The therapeutic effect of HNLI on head cancers was published in 1975 and 1981 (Ikić, D., et al. 1975; Ikić, D., et al. 1981; Padovan, I., 1981). The research we are now carring out is based on the aforementioned preliminary works.

Carcinoma basocellulare is infiltratable locally. It is made up of basal cells of the epidermis and is generally localised in the upper third of the face. The prognosis is unfavorable only in advanced cases, which may lead to the destruction of tissue all the way to the bone, invading vital structures or orifices (eyes, ears, bone, dura mater), to deformation and mutilation of often considerable size, and may cause death.

PATIENTS AND METHODS

Patients

Seventy-two patients with carcinoma basocellulare were treated with HNLI (Table 1). Forty-three patients were male and twenty-nine female, aged 25—88 years. The sites of tumors were: forehead — 10, face — 31, nose — 21, earshell — 5, retroauricular — 3, upper lip — 2.

RESULTS

Results of the HNLI therapy of 72 patients with ca. basocellulare are presented in Table 1. According to pathohistological and clinical findings, 52, patients out 72 (72.2%) were cured. Cancer lesions were completly epithelialized and cancer cells were not found in the bioptic material taken after HNLI treatment for the second biopsy. In 1 patient the cancer lesion was reduced more than 75% but cancer cells were found in the second biopsy. The cancer lesion was reduced about 50% in 4 patients, and was reduced about 25% in 4 patients. In 11 patients it was not reduced or was reduced less than 25%.

Results of the recombinant IFN alpha 2c in 12 patients with ca. basocellulare are presented in Table 2. According to pathohistological and clinical finding 5 patients out of 12 (42%) were cured. Cancer cells were not found in the bioptic material taken after recombinant IFN alpha 2c treatment and cancer lesions were completely epithelialized. In 4 patients out of 12 the cancer lesion was reduced more than 75%, and in 3 patients out of 12 the cancer lesion was reduced more than 50%.

Both types of interferons are very effective in the treatment of ca. basocellulare.

Results according to the localization of ca. basocellulare are presented in the next tables. Face localizations are presented in Table 3. The percentage of cured patients with the face localization of ca. basocellulare is 68% (19 out of 31). Nose localizations are presented in Table 4. The percentage of cured patients with the nose localization of ca. basocellulare is 76.2%, the percentage of patients with some reduction of cancer lesions is 4.8% (1 out of 21) and without any size reduction 19% (4 out of 21). Forehead localizations are presented in Table 5. The percentage of cured patients with forehead localizations is 90% (9 out of 10), the percentage of patients with some reduction of cancer lesions after the HNLI treatment is 10% (1 out of 10).

The last group of patients include ca. basocellulare localization on the earshell, retroauricular localization and localization on the lip. The percentage of cured patients with the aforementioned localizations is 50% (5 out of 10), the percentage of patients with some size reductions of the cancer lesions after HNLI treatment is 40% (4 out of 10) and without any size reduction 10% (1 out of 10).

DISCUSSION

The result of the interferon treatment of ca. basocellulare shows that it is possible to achieve the cure of patients in high proportion, and that the »cure« persists. Some patients have been followed more than 6 years so far.

Results of these studies confirm results published in our previous studies (Ikić, D., et al. 1975; Ikić, D., et al. 1981; Padovan, I., et al. 1981).

Greenway et al. (1986) treated 8 patients with biopsyproven ca. basocellulare. Patients were treated with recombinant IFN alpha 2c. They received intralesional injections of 1.5×10^6 units three times weekly for 3 weeks. Eight weeks after termination of the injections, the area of the lesions was

12 patients with carcinoma basocellulare were treated with recombinant HuIFN alpha 2c. 7 patients were male and 5 patients female, aged 49—86. The sites of tumors were: forehead — 4, nose — 5, face — 2, retroauricular left — 1 (Table 2).

Interferon

The duration of treatment with semipurified concentrated HNLI (10^4 units per 1 mg. protein) was 3—6 weeks. A single dose was 400.000—1,200,000 units. The number of applications ranged from 12—30 times, and the total dose was between 8—30 million units. The interferon as administered in 1 cc. with an intradermal needle (one injection but changing direction), causing both subtumoral and intratumoral infiltrations. No other medication was administered during the course of the interferon treatment. The HNLI was produced at the Institute of Immunology in Zagreb, Yugoslavia.

The duration of treatment with recombinant HuIFN alpha 2c was 4 weeks. Nine patients received 20 subtumoral and intratumoral injections of 2 million units (total dose: 40 million units). The recombinant HuIFN alpha 2c was applied daily except Saturday and Sunday. No other medication was administered during treatment. The recombinant HuIFN alpha 2c was produced by Boehringer Ingelheim and was donated to us free of charge.

Clinical evaluation of results:

0 = no effect

\+ = poor effect — tumor shows tendency towards regression (25%)

++ = satisfactory effect — size of tumor has significantly regressed (25—75%), clearly circumscribed

+++ = very good effect — size of tumor regressed more than 75%, or is present as barely visible nonepithelialized lesion

++++ = excellent effect — tumor has disappeared, fully epithelialized

Pathohistological evaluation of results:

0 = no response, non-reactive stroma

\+ = poor response, scattered lymphoid cells

++ = moderate response, numerous lymphoid cells, narrow infiltration areas, few macrophages

+++ = good response, massive infiltration of lymphoid cells, numerous macrophages

++++ = very good response, massive infiltration of lymphoid cells, numerous macrophages, tumorous cells have disappeared

excised and multiple slides were prepared from each tissue specimen. None of the tumors remained, even though the initial size ranged up to 14 × 12 mm. On histopathologic examination of the skin, all that indicated former abnormality were ectatic blood vessels, a mild perivascular infiltrate along with some incontinence of the pigment and Civatte body formation. (Greenway, H. J., et al. 1986). Their results confirm the results of our previous studies (Ikić, D., et al. 1975; Ikić, D., et al. 1981; Padovan, I., et a;l. 1981).

Photographs of patients and histopathological findings before and after treatment with interferon, show the efficacy of the therapy.

In our opinion the optimum dose is 2 — 5 × 10^6 units, depending on the size of the lesion, injected intralesionally, five times per week over 4 weeks. In the case of partial response or no response, the treatment can be prolonged up to 8 weeks with or without surgery.

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Table 1

HNLI. TUMOR OF THE HEAD AND NECK CA. BASOCELLULARE

PATIENT			Tumour site	Pathohistol. findings after treatment	Duration of treat. (weeks)	No. of applic	Single dose (x 10^6)	Total units (x 10^6)	Clinical grade
No.	Age	Sex							
1	52	M	Forehead	Sine tm.	3	15	1.2	18.0	++++
2	48	M	Face	Ca. basocellulare	6	30	0.4	12.0	0
3	42	F	Face	Sine tm.	5	25	0.4	10.0	++++
4	38	M	Nose	Sine tm.	5	24	0.4	9.6	++++
5	68	F	Face	Ca. basocellulare	6	30	0.4	12.0	+
6	79	F	Face	Sine tm.	5	20	0.8	16.0	++++
7	72	M	Left earshell	Ca. basocellulare	4	20	0.4	8.0	++
8	55	M	Nose	Sine tm.	6	30	0.5	15.0	++++
9	52	M	Nose	Sine tm.	6	30	0.5	15.0	++++
10	72	F	Nose	Sine tm.	6	30	0.5	15.0	++++
11	74	F	Nose	Sine tm.	6	30	0.5	15.0	++++
12	48	F	Forehead	Sine tm.	6	30	0.5	15.0	++++
13	64	M	Face	Sine tm.	6	30	0.5	15.0	++++
14	71	F	Face	Sine tm.	5	25	1.2	30.0	++++
15	25	M	Nose	Sine tm.	3	15	1.2	18.0	++++
16	63	M	Forehead	Sine tm.	5	24	0.5	12.0	++++
17	61	F	Face	Ca. basocellulare	4	18	0.5	9.0	+
18	68	M	Retroauricular	Sine tm.	4	18	1.2	21.6	++++
19	51	F	Forehead	Sine tm.	5	24	0.8	19.2	++++
20	51	F	Face	Sine tm.	5	24	0.8	19.2	++++
21	70	M	Face	Ca. basocellulare	3	12	0.8	9.6	+
22	64	M	Forehead	Sine tm.	5	24	0.8	19.2	++++
23	60	M	Nose	Sine tm.	5	24	0.8	19.2	++++
24	88	M	Face	Sine tm.	3	15	0.8	12.0	++++

PATIENT										
No.	Age	Sex	Tumour site	Pathohistol. findings after treatment	Duration of treat. (weeks)	No. of applic	Single dose (x 10^6)	Total units (x 10^6)	Clinical grade
25	76	M	Face	Sine tm.	5	24	0.8	19.2	++++
26	47	F	Retroauricular	Sine tm.	4	15	1.2	18.0	++++
27	86	F	Retroauricular	Ca. basocellulare	5	24	0.8	19.2	++
28	80	M	Face	Sine tm.	5	24	1.2	28.8	++++
29	48	F	Nose	Sine tm.	5	25	0.5	12.5	++++
30	37	M	Face	Sine tm.	4	20	0.5	10.0	++++
31	59	M	Face	Ca. basocellulare	5	25	0.5	12.5	0
32	72	F	Face	Sine tm.	4	20	0.8	16.0	++++
33	56	F	Face	Sine tm.	4	20	0.8	16.0	++++
34	71	M	Earshell	Ca. basocellulare	4	20	0.5	10.0	0
35	66	F	Earshell	Sine tm.	4	20	0.8	16.0	++++
36	56	M	Nose	Sine tm.	4	20	0.8	16.0	++++
37	66	M	Nose	Ca. basocellulare	5	25	0.8	20.0	0
38	46	M	Nose	Sine tm.	4	20	0.8	16.0	++++
39	53	F	Nose	Sine tm.	4	20	1.0	20.0	++++
40	78	M	Forehead	Sine tm.	4	20	1.0	20.0	++++
41	76	F	Nose	Sine tm.	4	20	1.0	20.0	++++
42	58	M	Face	Sine tm.	4	20	1.0	20.0	++++
43	75	M	Forehead	Sine tm.	4	20	1.0	20.0	++++
44	55	M	Face	Sine tm.	4	20	1.0	20.0	++++
45	42	M	Face	Ca. basocellulare	4	20	1.0	20.0	0
46	68	M	Upper lip	Sine tm.	4	20	1.0	20.0	++++
47	65	F	Face	Sine tm.	4	20	1.0	20.0	++++
48	75	M	Face	Ca. basocellulare	4	20	1.0	20.0	0

PATIENT										
No.	Age	Sex	Tumour site	Pathohistol. findings after treatment	Duration of treat. (weeks)	No. of applic	Single dose (x 10^6)	Total units (x 10^6)	Clinical grade
49	64	F	Forehead	Sine tm.	4	20	1.0	20.0	++++
50	83	M	Nose	Ca. basocellulare	4	20	1.0	20.0	0
51	58	M	Face	Sine tm.	4	20	1.0	20.0	++++
52	65	F	Face	Sine tm.	4	20	1.0	20.0	++++
53	83	M	Nose	Ca. basocellulare	4	20	1.0	20.0	0
54	75	M	Forehead	Sine tm.	4	20	1.0	20.0	++++
55	42	M	Face	Ca. basocellulare	4	20	1.0	20.0	0
56	76	F	Nose	Sine tm.	4	20	1.0	20.0	++++
57	53	F	Nose	Sine tm.	4	20	1.0	20.0	++++
58	55	M	Face	Ca. basocellulare	4	20	1.0	20.0	+++
59	63	M	Face	Ca. basocellulare	4	20	0.5	10.0	++
60	59	M	Forehead	Sine tm.	4	20	1.0	20.0	++++
61	65	F	Face	Ca. basocellulare	6	30	0.4	12.0	+
62	66	F	Earshell	Sine tm.	4	20	0.8	16.0	++++
63	66	M	Nose	Sine tm.	4	20	0.8	16.0	++++
64	72	F	Face	Sine tm.	4	20	0,8	16,0	++++
65	66	M	Nose	Ca. basocellulare	5	25	0.8	20.0	0
66	59	M	Face	Sine tm.	4	20	0.5	10.0	++++
67	79	M	Face	Ca. basocellulare	4	20	1.0	20.0	0
68	86	F	Earshell	Ca. basocellulare	5	24	0.8	19.2	++
69	56	M	Upper lip	Sine tm.	4	20	1.0	20.0	++++
70	56	F	Face	Sine tm.	4	20	0.8	16.0	++++
71	48	F	Nose	Sine tm.	5	25	0.5	12.5	++++
72	56	M	Nose	Sine tm.	5	24	0.4	9.6	++++

Table 2

R. IFN alpha 2c TUMOURS OF THE HEAD AND NECK CA. BASOCELLULARE

PATIENT										
No.	Age	Sex	Tumor site	Pathohistol. findings after treatment	Duration of treat. (weeks)	No. of applic.	Single dose (x 10^6)	Total units (x 10^6)	Clinical grade
1	86	M	Forehead	Sine tm.	4	20	2.0	40.0	++++
2	53	F	Forehead	Ca basocellulare	4	20	2.0	40.0	++
3	49	M	Forehead	Ca basocellulare	4	20	2.0	40.0	++
4	62	F	Face	Sine tm.	4	20	2.0	40.0	++++
5	61	F	Forehead	Sine tm.	4	20	2.0	40.0	++++
6	63	M	Face	Sine tm.	4	20	2.0	40.0	++++
7	66	F	Nose	Sine tm.	4	20	2.0	40.0	++++
8	70	F	Nose	Ca. basocellulare	4	20	2.0	40.0	+++
9	68	M	Nose	Ca. planocellulare	4	20	2.0	40.0	++
10	73	M	Retroauricular	Ca. basocellulare	4	20	5.0	100.0	++++
11	74	M	Nose	Ca. basocellulare	4	20	5.0	100.0	+++
12	70	M	Nose	Ca. basocellulare	4	20	5.0	100.0	++++

Table 3

HNLI. TUMOURS OF THE HEAD AND NECK CA. BASOCELLULARE — FACE

PATIENT										
No.	Age	Sex	Tumour site	Pathohistol. findings afte treatment	Duration of treat. (weeks)	No. of applic.	Single dose (x 10^6)	Total units (x 10^6)	Clinical grade
1	71	F	Face	Sine tm.	5	25	1.2	30.0	++++
2	80	M	Face	Sine tm.	5	24	1.2	28.8	++++
3	58	M	Face	Sine tm.	4	20	1.0	20.0	++++
4	58	M	Face	Sine tm.	4	20	1.0	20.0	++++
5	55	M	Face	Sine tm.	4	20	1.0	20.0	++++
6	65	F	Face	Sine tm.	4	20	1.0	20.0	++++
7	65	F	Face	Sine tm.	4	20	1.0	20.0	++++
8	60	M	Face	Sine tm.	5	24	0.8	19.2	++++
9	25	M	Face	Sine tm.	5	24	0.8	19.2	++++
10	79	F	Face	Sine tm.	5	20	0.8	16.0	++++
11	72	F	Face	Sine tm.	4	20	0.8	16.0	++++
12	56	F	Face	Sine tm.	4	20	0.8	16.0	++++
13	72	F	Face	Sine tm.	6	30	0.5	15.0	++++
14	56	M	Face	Sine tm.	6	30	0.5	15.0	++++
15	64	M	Face	Sine tm.	5	24	0.8	19.2	++++
16	88	F	Face	Sine tm.	4	18	1.2	21.6	++++
17	42	M	Face	Sine tm.	5	24	0.5	12.0	++++
18	37	F	Face	Sine tm.	3	15	1.2	18.0	++++
19	57	M	Face	Sine tm.	4	20	0.5	10.0	++++
20	55	M	Face	Ca. basocellulare	4	20	1.0	20.0	+++
21	63	M	Face	Ca. basocellulare	4	20	0.5	10.0	++
22	68	F	Face	Ca. basocellulare	6	30	0.4	12.0	+
23	65	F	Face	Ca. basocellulare	6	30	0.4	12.0	+
24	70	M	Face	Ca. basocellulare	3	12	0.8	9.6	+
25	61	F	Face	Ca. basocellulare	4	18	0.5	9.0	+
26	42	M	Face	Ca. basocellulare	4	20	1.0	20.0	0
27	75	M	Face	Ca. basocellulare	4	20	1.0	20.0	0
28	42	M	Face	Ca. basocellulare	4	20	1.0	20.0	0
29	79	M	Face	Ca. basocellulare	4	20	1.0	20.0	0
30	59	M	Face	Ca. basocellulare	5	25	0.5	12.5	0
31	48	M	Face	Ca. basocellulare	6	30	0.4	12.0	0

Table 4

HNLI. TUMOURS OF THE HEAD AND NECK CA. BASOCELLULARE — NOSE

PATIENT			Tumour site	Pathohistol. findings after treatment	Duration of treat. (weeks)	No. of applic.	Single dose (x 10^6)	Total units (x 10^6)	Clinical grade
No.	Age	Sex							
1	53	F	Nose	Sine tm.	4	20	1.0	20.0	++++
2	53	F	Nose	Sine tm.	4	20	1.0	20.0	++++
3	76	F	Nose	Sine tm.	4	20	1.0	20.0	++++
4	76	F	Nose	Sine tm.	4	20	1.0	20.0	++++
5	60	M	Nose	Sine tm.	5	24	0.8	19.2	++++
6	25	M	Nose	Sine tm.	3	15	1.2	18.0	++++
7	56	M	Nose	Sine tm.	4	20	0.8	16.0	++++
8	46	M	Nose	Sine tm.	4	20	0.8	16.0	++++
9	66	M	Nose	Sine tm.	4	20	0.8	16.0	++++
10	55	M	Nose	Sine tm.	6	30	0.5	15.0	++++
11	52	M	Nose	Sine tm.	6	30	0.5	15.0	++++
12	72	F	Nose	Sine tm.	6	30	0.5	15.0	++++
13	74	F	Nose	Sine tm.	6	30	0.5	15.0	++++
14	48	F	Nose	Sine tm.	5	25	0.5	12.5	++++
15	48	F	Nose	Sine tm.	5	25	0.5	12.5	++++
16	56	M	Nose	Sine tm.	5	24	0.4	9.6	++++
17	38	M	Nose	Ca. basocellulare	5	24	0.4	9.6	+++
18	66	M	Nose	Ca. basocellulare	5	25	0.8	20.0	0
19	66	M	Nose	Ca. basocellulare	5	25	0.8	20.0	0
20	83	M	Nose	Ca. basocellulare	4	20	1.0	20.0	0
21	83	M	Nose	Ca. basocellulare	4	20	1.0	20.0	0

Table 5

HNLI. TUMOURS OF THE HEAD AND NECK CA. BASOCELLULARE — FOREHEAD

PATIENT			Tumour site	Pathohistol. findings after treatment	Duration of treat. (weeks)	No. of applic.	Single dose (x 10^6)	Total units (x 10^6)	Clinical grade
No.	Age	Sex							
1	78	M	Forehead	Sine tm.	4	20	1.0	20.0	++++
2	64	F	Forehead	Sine tm.	4	20	1.0	20.0	++++
3	75	M	Forehead	Sine tm.	4	20	1.0	20.0	++++
4	59	M	Forehead	Sine tm.	4	20	1.0	20.0	++++
5	51	F	Forehead	Sine tm.	5	24	0.8	19.2	++++
6	64	M	Forehead	Sine tm.	5	24	0.8	19.2	++++
7	48	F	Forehead	Sine tm.	6	30	0.5	15.0	++++
8	63	M	Forehead	Sine tm.	5	24	0.5	12.0	++++
9	75	M	Forehead	Ca. basocellulare	4	20	1.0	20.0	++
10	52	M	Forehead	Sine tm.	3	15	1.2	18.0	++++

Table 6

HNLI. TUMOURS OF THE HEAD AND NECK CA. BASOCELLULARE — EARSHELL, RETROAURICULAR AND LIP

PATIENT			Tumour site	Pathohistol. findings after treatment	Duration of treat. (weeks)	No. of applic.	Single dose (x 10^6)	Total units (x 10^6)	Clinica grade
No.	Age	Sex							
1	66	F	Earshell	Sine tm.	4	20	0.8	16.0	++++
2	66	F	Earshell	Ca. basocellulare	4	20	0.8	16.0	+++
3	86	F	Earshell	Ca. basocellulare	5	24	0.8	19.2	++
4	72	M	Earshell	Ca. basocellulare	4	20	0.4	8.0	++
5	71	M	Earshell	Ca. basocellulare	4	20	0.5	10.0	0
6	68	M	Retroauricular	Sine tm.	4	18	1.2	21.6	++++
7	47	F	Retroauricular	Sine tm.	4	15	1.2	18.0	++++
8	86	F	Retroauricular	Ca. basocellulare	5	24	0.8	19.2	++
9	68	M	Upper lip	Sine tm.	4	20	1.0	20.0	++++
10	56	M	Upper lip	Sine tm.	4	20	1.0	20.0	++++

SUMMARY

Results of the HNLI therapy of 72 patients with ca. basocellulare are presented in Table 1. According to pathohistological and clinical findings 52 patients out of 72 (72,2%) were cured. Cancer lesions were completely epitelialized and cancer cells were not found in the bioptic material taken after HNLI treatment for the second biopsy. In 1 patient cancer lesion was reduced more than 75% but the cancer cells were found in the second biopsy. Cancer lesion was reduced about 50% in 4 patients and it was reduced about 25% in 4 patients. In 11 patients was not reduced or it was less than 25% reduced.

Results of the recombinant IFN alpha 2c therapy of 12 patients with ca. basocellulare are presented in Table 2. According to pathohistological and clinical finding 5 patients out of 12 (42%) were cured. Cancer cells were not found in the bioptic material taken after recombinant IFN alpha 2c treatment and cancer lesions were completely epitelialized.

In 4 patients out of 12 cancer lesion was reduced more than 75% and in 3 patients out of 12 cancer lesion was reduced more than 50%.

Both types of interferons are very effective in the treatment of ca. basocellulare.

SAŽETAK

APLIKACIJA INTERFERONA KOD BOLESNIKA SA BAZEOCELULARNIM KARCINOMOM

Rezultati terapije humanim leukocitnim interferonom 72 pacijenta s dijagnozom ca. basocellulare prikazani su u tablici 1. Prema patohistološkom i kliničkom nalazu 52 od 72 (72.2) pacijenta su izliječeni. Lezije tumora kompletno su epitelizirane i stanice tumora nisu nađene u bioptičkom materijalu uzetom nakon tretmana humanim leukocitnim interferonom. Kod 1 pacijenta lezije tumora smanjene su više od 75%. Kod 4 pacijenta lezije su smanjene oko 50% i kod 4 pacijenta smanjene su oko 25%. Kod 11 pacijenata lezije nisu smanjene ili su smanjene manje od 25%.

Rezultati terapije rekombinantnim interferonom alpha 2c kod 12 pacijenata s dijagnozom ca. basocellulare prikazani su u tabeli 2. Prema patohistološkom i kliničkom nalazu 5 od 12 pacijenata (42%) izliječeno je. Stanice tumora nisu nađene u bioptičkom materijalu uzetom nakon tretmana rekombinantnim interferonom alpha 2c i lezije su u potpunosti epitelizirane.

Kod 4 pacijenta od 12 lezije su smanjene više od 75%, a kod 3 pacijenta od 12 smanjene su više od 50%. Oba tipa interferona vrlo su efikasna u liječenju ca. basocellulare.

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APPLICATION OF INTERFERON IN PATIENTS WITH CARCINOMA PLANOCELLULARE

Interferons are a family of naturally occuring biomodulators, induced in cells by a variety of viral, bacterial, syntetic and other stimulants. They have, besides antiviral activities as originally discovered by Isaacs and Lindeman (1957), immunoregulatory and antiproliferative activities. The inhibiton of tumour virus replication, the concommitant cell transformation and the inhibition of cell growth are of particular oncological interest. Interferons are classified on the basis of their antigenic, biological and chemical properties into three major groups designated interferon alpha, -beta and -gamma. Two of them, alpha and beta, appear to be related and are acid stable. Interferon gamma is a natural product of lymphocyte populations especially from interleukin 2 stimulated T-lymphocytes. Interferon gamma (immune interferon) is acid labile and induced in response to stimulation of specific mitogens or antigens.

The »natural« human leucocyte interferons were originaly harvested from white blood cells stimulated by viruses. This product is less than 1% pure and contains other lymphokines. The production of »natural« interferons is limited by the white blood cell supply. This disadvantage was overcome with the advent of recent bioengineering, allowing for the production of sufficent and uniform quantities of recombinant interferons. Over 20 subtypes of interferon alpha have been indentified, the most biologically important being interferon alpha 2. Recombinant molecular technology has led to the insertion of human DNA sequences coding for interferon alpha 2 into Escherichia coli, which then express large quantities of interferon alpha 2, which is again purified by chromatography. Recombinant interferon alpha 2 is more than 99% pure.

Carcinoma planocellulare is an epidermal invasive cancer of the skin and visible mucous membranes, made up of spinous epidermal cells. A high precentage of undifferentiated cells without keratotic pearls indicates malignancy of the tumour, which ulcerates and invades the underlying tissue. About 1/3 of lingual and mucosal lesions have metastasized before they are diagnosed.

Our first results with peritumoural and intratumoural application of human natural leucocytic interferon (HNLI) in patients with carcinoma planocellulare were published in 1981 (Ikić et al. 1981; Padovan et al. 1981). On the basis of the second pathohistological biopsy taken immediately following the end of therapy from the site where the carcinoma had been located, results were negative in 6 of the 8 cases. In the research we are now carring out, HNLI and recombinant HuIFN alpha 2c are used in the therapy of carcinoma planocellulare.

PATIENTS AND METHODS

Patients:

Thirty-two patients with carcinoma planocellulare were treated with HNLI (Table 1). Nineteen patients were male and thirteen female, aged 26—87 years. The sites of the tumours were: lower lip — 11, upper lip — 2, tonsils — 2, forehead — 4, nose — 5, earshell — 7, retroauricular — 1. Ten patients with carcinoma planocellulare were treated with recombinant HuIFN alpha 2c (Table 2). Seven patients were male and three female, aged 50—81. The sites of the tumours were: lower lip — 6, forehead — 1, face — 1, skin of ear — 1, nose — 1.

Interferon:

The duration of treatment with semipurified concentrated HNLI (10^4 units per 1 mg. protein) was 3—6 weeks. A single dose was 400,000—1,200,000 units. The number of applications ranged from 12—30 times and the total dose was between 6—21.6 million units. The interferon was administered in 1cc. with an intradermal needle (one injection but changing direction), causing both subtumoural and intratumoural infiltration. No other medication was administered during the course of the interferon treatment. The HNLI was produced at the Institute of Immunology in Zagreb, Yugoslavia.

The duration of treatment with recombinant HuIFN alpha 2c was 4 weeks. Six patients received 20 subtumoural and intratumoural injection of 2,000,000 units of r.IFN alpha 2c (total dose: 40 million units). The recombinant IFN alpha 2c was applied daily except for Saturday and Sunday. No other medication was administered during treatment. The recombinant IFN alpha 2c was produced by Boehringer Ingelheim and was donated to us free of charge.

Pathohistological evaluation of results:

0 = no response, non-reactive stroma
\+ = poor response, scattered lymphoid cells
++ = moderate response, numerous lymphoid cells, narrow infiltration areas, few macrophages
+++ = good response, massive infiltration of lymphoid cells, numerous macrophages

++++ = very good response, massive infiltration of lymphoid cells, numerous macrophages, tumorous cells have disappeared

Clinical evaluation of results:

0 = no effect
\+ = poor effect — tumor shows tendency towards regression (25%)
++ = satisfactory effect — size of tumor has significantly regressed (25—75%), clearly circumscribed
+++ = very good effect — size of tumor regressed more than 75%, or is present as barely visible nonepithelialized lesion
++++ = excellent effect — tumor has disappeared, fully epithelialized

RESULTS

Results of the human natural leucocytic interferon therapy of 32 patients with ca. planocellulare are presented in table 1. According to pathohistological and clinical findings 19 out of 32 patients (nos. 2, 4, 5, 6, 7, 10, 11, 12, 13, 14, 15, 19, 20, 21, 23, 25, 27, 31, 32) were cured and tumour cells were not found in the material taken after interferon treatment for the second biopsy. In two patients (nos. 17 and 30) the tumour size was reduced more than 75% according to clinical findings. In seven patients (nos. 1, 3, 8, 9, 18, 26, 29) the tumour size was reduced more than 50%, in one patient (no. 16) the tumour size was reduced about 25%, and in three patients (nos. 22, 24, 28) the tumour size was not reduced according to clinical findings.

Results of the human natural leucocytic interferon therapy of 15 patients with ca. planocellulare of the lower lip, upper lip, and tonsils are presented in table 3. These tumours often leave the patient disfigured after surgery. Five of the eleven patients with lower lip ca. planocellulare were considered cured according to pathohistological findings (nos. 1—5). In one patient (no. 6), there was a reduction in tumour size more than 75%, in four patients (nos. 7, 8, 9, 10) tumour size was reduced more than 50%, and in one patient (no. 11) there was no reduction in tumour size according to clinical findings. One of two patients with tonsil ca. planocellulare (no. 12) was considered cured according to pathohistological findings. In one patient (no. 13), there was reduction in tumour size more than 50% according to clinical findings. In two patients with upper lip ca. planocellulare (nos. 14, 15), the tumour size was not reduced according to clinical findings.

The site of the other seventeen ca. planocellulare were: earshell, nose, forehead, and retroauricular. Results of the therapy are presented in table 4. Six out of seven patients with earshell ca. planocellulare were considered cured according to pathohistological findings (nos. 1—6). In one patient (no. 7) there was reduction in size more than 50% according to clinical finding.

Two of five patients with nose ca. planocellulare were considered cured according to pathohistological findings (nos. 8, 9). In one patient (no. 10) there was reduction in size more than 75%, in one patient (no. 11) there was reduction in size more than 50% according to clinical findings.

All four patients with forehead ca. planocellulare and one retroauricular ca. planocellulare were considered cured according to pathohistological findings (nos. 13, 14, 15, 16, 17). Results of the recombinant HuIFN alpha 2c therapy are summarized in Table 2. Four out of ten patients with ca. planocellulare (nos. 2, 5, 7, 8) were cured according to pathohistological and clinical findings. In two patients (nos. 3 and 10) there was a reduction in tumour size more than 75%, in one patient (no. 9) tumour size was reduced more than 50%, and in two patients (nos. 1 and 6) there was a 25% reduction in tumour size.

Only one of six patients with lower lip ca. planocellulare was considered cured according to pathohistological and clinical findings (no. 8). In two patients (nos. 3 and 10) there was a reduction in tumour size more than 75%, in one patient (no. 9) the tumour size was reduced more than 50%, and in two patients (nos. 1 and 6) there was a 25% reduction in tumour size. In one patient there was no reduction in tumour size (no. 4).

Four other patients had different sites of the tumour (forehead, face, nose, ear). Three out of four patients were considered cured according to pathohistological and clinical findings (nos. 2, 5 and 7). In one patient (no. 4) there was no reduction in tumour size.

DISCUSSION

Intralesional injection in the tumours was introduced by Ikić D. and Ikić D. et al. and published in 1975. Injection of interferon into a skin tumour is simple and harmless. The interferon titer of the injected lesion is higher and the higher titer is responsible for the enhanced local antitumor activity. A highly significant difference between intralesional and systemic responses proves that the local antitumor effect of interferon is superior to the systemic effect (Wussov, P. et al. 1988). Intralesional injections of patients with basal cell carcinoma with interferon alpha 2b were performed succesfully by Greenway H. T. et al. (1986).

A small concentration of units is required if injected intralesionally, and the incidence of interferon side effects is lower. A massive systemic injection of interferon is accompanied by a flu-like syndrome which is due to the interferon level in the plasma. The treatment toxicities are dose dependent. High doses may regularly be associated with respiratory depression, confusion, depression in the level of consciousness and altered mental status. Fortunately these are less apparent with conventional doses (Roche laboratories, Physicians Desk Reference, 1987; Schering Corporation, Physicians Desk Reference, 1987). There are no side effects after 2 million units of interferon injected intralesionally, and after 5 million units, side reactions are very mild. Even if interferon enters the plasma, the small concentration of interferon and very efficient catabolic system prevents side reactions. Local production of interferon as the production of other lympkokines can be called a »physiological response« which has an important role in the immune system (Bocci, V., 1987; Bocci, V., 1985; Bocci, V., 1981).

After massive interferon administration, the blood levels reach their maximum concentration within 3—4 hours. The half-life of elimination ranges

from 6.0—12.0 hours, with the principal route of catabolism and excretion being the kidney.

Preliminary reports of an overall incidence of neutralizing antibodies in 17 per cent of recipients after massive interferon administration seems far in excess of the current situation. Recent assessment places their incidence at less than 5 per cent. There are fears that production of neutralizing antibodies would dampen the response to interferon.

Interferon is a great promise for patients with ca. planocellulare (squamous cell carcinoma) especially in surgically and cosmetically difficult and troubling locations. This mode of therapy is welcome compared with destructive surgical techniques.

Our data clearly show that interferon intralesional therapy of ca. planocellulare is very effective. According to pathohistological and clinical findings 19 out 32 patients were cured with intralesional injection of the human natural leucocytic interferon. In 10 patients, tumor size was significantly reduced, and in 3 patients, tumor size was not reduced according to clinical findings. Four out of ten patients were cured with intralesional injection of the recombinant HuIFN alpha 2c, and in the other 5 patients tumor size was significantly reduced. In cosmetically difficult and surgically troubling locations intralesional interferon therapy can be considered front-line therapy, in order to avoid disfigurement of the patients, and either combined with of without surgery. The response rate is very high more than 90%, and the complete response rate is more than 50%.

In our opinion the optimum dose is $2—5 \times 10^6$ units depending on the size of lesion. The recomanded route of administration in intralesional and the schedule of therapy 5 times per week, with duration of treatment 4 weeks. In the case of partial response, the treatment can be prolonged up to 8 weeks with or without surgery.

The question whether combination of different types of interferons will result in a synergistic anti-tumour effect has to be considered. Laboratory studies of squamous-cell carcinomas in mice have revealed that a statistically significant improvement occured with the combination of all three types of interferons (Brysk, M. M., et al. 1981).

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SUMMARY

Results of the human natural leucocytic interferon therapy of 32 patients with ca. planocellulare are presented in Table 1. According to pathohistological and clinical findings 19 out 32 patients (nos. 2, 4, 5, 6, 7, 10, 11, 12, 13, 14, 15, 19, 20, 21, 23, 25, 27, 31, 32) were cured and tumour cells were not found in the material taken after interferon treatment for the second biopsy. In two patients (nos. 17, 30) tumour size was reduced more than 75% according to clinical findings. In seven patients (nos. 1, 3, 8, 9, 18, 26, 29) tumour size was reduced more than 50%, in one patient (no. 16) tumour size was reduced about 25%, and in three patients (nos. 22, 24, 28) tumour size was not reduced according to clinical findings.

Results of the recombinant HuIFN alpha 2c therapy are summarized in Table 2. Four out of ten patients with ca. planocellulare (nos. 2, 5, 7, 8) were cured according to pathohistological and clinical findings. In two patients (nos. 3 and 10) there was a reduction in tumour size more than 75%, in one patient (no. 9) tumour size was preduced more than 50% and in two patients (nos. 1 and 6) there was 25% reduction in tumour size.

SAŽETAK

APLIKACIJA INTERFERONA KOD BOLESNIKA SA PLANOCELULARNIM KARCINOMOM

Rezultati liječenja humanim prirodnim leukocitnim interferonom 32 pacijenta oboljela od planocelularnog karcinoma prikazani su u tablici 2. Slijedom patohistološkog i kliničkog nalaza 19 od 32 pacijenta (brojevi 2, 4, 5, 6, 7, 10, 11, 12, 13, 14, 15, 19, 20, 21, 23, 25, 27, 31, 32) izliječeni su i nisu nađene tumorske stanice u materijalu uzetom u drugoj biopsiji nakon tretmana interferonom. Kod dva pacijenta (broj 17, 30) veličina tumora smanjena je više od 75% prema kliničkom nalazu. Kod 7 pacijenata (br. 1, 3, 8, 9, 18, 26, 29) veličina tumora smanjena je više od 50%, kod jednog pacijenta (br. 16) veličina tumora smanjena je oko 25%, a kod tri pacijenta (br. 22, 24, 28) veličina tumora nije smanjena prema kliničkom nalazu.

Table 1

HNLI. TUMOUR OF THE HEAD AND NECK CA. PLANOCELLULARE

PATIENT			Tumour site	Pathohistol. findings after treatment	Duration of treat. (weeks)	No. of applic.	Single dose (x 10^6)	Total units (x 10^6)	Clinical grade
No.	Age	Sex							
1	78	M	Lower lip	Ca. planocellulare	5	24	0.8	19.2	++
2	58	M	Lower lip	Sine tm.	4	18	1.2	21.6	++++
3	50	M	Left tonsil	Ca. planocellulare	5	24	0.5	12.0	++
4	41	F	Right tonsil	Sine tm.	3	14	0.4	5.6	++++
5	60	F	Forehead	Sine tm.	3	12	0.8	9.6	++++
6	34	F	Lower lip	Sine tm.	4	16	0.5	8.0	++++
7	62	M	Forehead	Sine tm.	4	15	0.8	12.0	++++
8	50	F	Lower lip	Ca. planocellulare	5	20	0.4	8.0	++
9	66	F	Nose	Ca. planocellulare	4	20	0.5	10.0	++
10	42	M	Left earshell	Sine tm.	5	18	0.4	7.2	++++
11	43	M	Right earshell	Sine tm.	5	15	0.4	6.0	++++
12	48	M	Nose	Sine tm.	6	30	0.5	15.0	++++
13	55	M	Nose	Sine tm.	6	30	0.5	15.0	++++
14	26	F	Earshell	Sine tm.	6	30	0.5	15.0	++++
15	47	M	Forehead	Sine tm.	5	24	0.8	19.2	++++
16	63	F	Nose	Ca. planocellulare	4	18	1.2	21.6	+
17	74	M	Lower lip	Ca. planocellulare	5	24	0.5	12.0	+++
18	70	F	Lower lip	Ca. planocellulare	3	15	1.2	18.0	++
19	68	M	Earshell	Sine tm.	4	18	1.2	21.6	++++
20	87	M	Retroauricular	Sine tm.	4	15	0.4	6.0	++++
21	35	M	Forehead	Sine tm.	6	30	0.5	15.0	++++
22	50	M	Lower lip	Ca. planocellulare	3	15	0.8	12.0	0
23	53	M	Lower lip	Sine tm.	4	20	1.0	20.0	++++
24	63	F	Upper lip	Ca. planocellulare	4	20	1.0	20.0	0
25	64	F	Lower lip	Sine tm.	4	16	0.5	8.0	++++

PATIENT No.	Age	Sex	Tumour site	Pathohistol. findings after treatment	Duration of treat. (weeks)	No. of applic.	Single dose (x 10^6)	Total units (x 10^6)	Clinical grade
26	76	M	Earshell	Ca. planocellulare	4	20	0.4	8.0	++
27	49	M	Lower lip	Sine tm.	3	15	0.8	12.0	++++
28	63	F	Upper lip	Ca. planocellulare	4	20	1.0	20.0	0
29	66	F	Lower lip	Ca. planocellulare	4	20	0.5	10.0	++
30	66	F	Nose	Ca. planocellulare	4	20	0.5	10.0	+++
31	41	M	Earshell	Sine tm.	5	18	0.4	7.2	++++
32	78	M	Earshell	Sine tm.	4	15	0.8	12.0	++++

Table 2

R.IFN alpha 2c TUMOURS OF THE HEAD AND NECK CA. PLANOCELLULARE

PATIENT No.	Age	Sex	Tumour site	Pathohistol. findings after treatment	Duration of treat. (weeks)	No. of applic.	Single dose (x 10^6)	Total units (x 10^6)	Clinical grade
1	55	M	Lower lip	Ca. planocellulare	4	20	2.0	40.0	+
2	50	F	Forehead	Sine tm.	4	20	2.0	40.0	++++
3	56	M	Lower lip	Ca. planocellulare	4	20	2.0	40.0	+++
4	68	F	Face	Ca. planocellulare	4	20	2.0	40.0	0
5	53	M	Earshell	Sine tm.	4	20	2.0	40.0	++++
6	81	F	Lower lip	Ca. planocellulare	4	20	2.0	40.0	+
7	66	M	Nose	Sine tm.	4	20	5.0	100.0	++++
8	64	M	Lower lip	Sine tm.	4	20	5.0	100.0	++++
9	54	M	Lower lip	Ca. planocellulare	4	20	5.0	100.0	++
10	38	M	Lower lip	Ca. planocellulare	4	20	5.0	100.0	+++

Rezultati terapije rekombinantnim HuIFN alpha 2c prikazani su u tablici 2. Četiri od 10 pacijenata s planocelularnim karcinomom (br. 2, 5, 7, 8) izliječeni su prema patohistološkom i kliničkom nalazu. Kod dva pacijenta (br. 3 i 10) smanjena je veličina tumora više od 75%, kod dva pacijenta (br.4 i 9) veličina tumora smanjena je više od 50% i kod dva pacijenata (br. 1 i 6) veličina tumora smanjena je 25%.

Table 3

HNLI. TUMOUR OF THE HEAD AND NECK CA. PLANOCELLULARE — LIP and TONSIL

PATIENT No.	Age	Sex	Tumour site	Pathohistol. findings after treatment	Duration of treat. (weeks)	No. of applic.	Single dose (x 10^6)	Total units (x 10^6)	Clinical grade
1	53	M	Lower lip	Sine tm.	4	20	1.0	20.0	++++
2	49	M	Lower lip	Sine tm.	3	15	0.8	12.0	++++
3	34	F	Lower lip	Sine tm.	4	16	0.5	8.0	++++
4	64	F	Lower lip	Sine tm.	4	16	0.5	8.0	++++
5	58	M	Lower lip	Sine tm.	4	18	1.2	21.6	++++
6	74	M	Lower lip	Ca. planocellulare	5	24	0.5	12.0	+++
7	78	M	Lower lip	Ca. planocellulare	5	24	0.8	19.2	++
8	70	F	Lower lip	Ca. planocellulare	3	15	1.2	18.0	++
9	66	F	Lower lip	Ca. planocellulare	4	20	0.5	10.0	++
10	50	F	Lower lip	Ca. planocellulare	5	20	0.4	8.0	++
11	50	M	Lower lip	Ca. planocellulare	3	15	0.8	12.0	0
12	41	F	Right Tonsil	Sine tm.	3	14	0.4	5.6	++++
13	50	M	Left tonsil	Ca. planocellulare	5	24	0.5	12.0	++
14	63	F	Upper lip	Ca. planocellulare	4	20	1.0	20.0	0
15	63	F	Upper lip	Ca. planocellulare	4	20	1.0	20.0	0

Table 4

HNLI. TUMOURS OF THE HEAD AND NECK CA. PLANOCELLULARE — EARSHELL, NOSE and FOREHEAD

PATIENT			Tumour site	Pathohistol. findings after treatment	Duration of treat. No. of (weeks) applic.		Single dose (x 10^6)	Total units (x 10^6)	Clinical grade
No.	Age	Sex							
1	68	M	Earshell	Sine tm.	4	18	1.2	21.6	++++
2	26	F	Earshell	Sine tm.	6	30	0.5	15.0	++++
3	78	M	Earshell	Sine tm.	4	15	0.8	12.0	++++
4	42	M	Earshell	Sine tm.	5	18	0.5	7.2	++++
5	41	M	Earshell	Sine tm.	5	18	0.4	7.2	++++
6	43	M	Earshell	Sine tm.	5	15	0.4	6.0	++++
7	76	M	Earshell	Ca. planocellulare	4	20	0.4	8.0	++
8	48	M	Nose	Sine tm.	6	30	0.5	15.0	++++
9	55	M	Nose	Sine tm.	6	30	0.5	15.0	++++
10	66	F	Nose	Ca. planocellulare	4	20	0.5	10.0	+++
11	66	F	Nose	Ca. planocellulare	4	20	0.5	10.0	++
12	63	F	Nose	Ca. planocellulare	4	18	1.2	21.6	+
13	47	M	Forehead	Sine tm.	5	24	0.8	19.2	++++
14	35	M	Forehead	Sine tm.	6	30	0.5	15.0	++++
15	62	M	Forehead	Sine tm.	4	15	0.8	12.0	++++
16	60	F	Forehead	Sine tm.	3	12	0.8	9.6	++++
17	87	M	Retroauricular	Sine tm.	4	15	0.4	6.0	++++

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MELANOMAS. THERAPY WITH RECOMBINANT INTERFERON HUIFN ALPHA 2C. PRELIMINARY REPORT.

Melanoma, once considered a rare form of cancer, is increasing in incidence throughout the world.

The use of interferon as an anticancer agent in melanoma stems from the work of Ratner L. et al. (1980), who showed the inhibition of growth in mouse melanoma, the influence on the antigen expression of melanoma cells (Liao, S. K., 1982), and on a whole variety of immunoregulatory effects on the hosts immunosurveillance (De Maeyer Guignard, J., 1986).

The aim of this research is to establish the effect of recombinant interferon alpha 2c in melanomas. Since we had not had experience with melanoma until now, we applied the similar technique and duration of interferon application which had led to clinical cure in more than 50% of ca. basocellulare and ca. planocellulare cases (Ikić, D., et al. 1981; Padovan, I., et al. 1981).

MATERIALS AND METHODS

Patients

Thirteen melanoma patients were treated with recombinant HuIFN alpha 2c (Table 1). Nine patients were female and four male, aged 30—78 years. The sites of the tumours were: dorsal 1, neck 3, anocutaneous border 1, left axilla 1, right upper arm 1, right lower arm 1, right lower leg 1, planta of right leg 1, right upper leg 1, left upper leg 1, left lower leg 1.

In 4 cases the melanoma patients received interferon only after surgery (nos. 1, 7, 9, 13), and in 9 cases, before suregical procedure was done (nos. 2, 3, 4, 5, 6, 8, 10, 11, 12). The size of the melanomas, according to the longer diameter, was from 8 mm. to 56 mm. and according to the shorter diameter, from 8 mm. to 50 mm. Clinical grade on basis of anamnesis: $T_1N_0M_0 = 4$, $T_2N_0M_0 = 1$, $T_3N_0M_0 = 3$, $T_3N_1M_0 = 4$.

Interferon

Recombinant HuIFN alpha 2c was applied peritumorally, subtumourally and intratumourally. The duration of treatment was 1 day — 4 weeks. The

number of application was 1—20. Interferon was not administered on Saturday and Sunday. A single dose was 2 million units and the total dose, 40 million units in seven cases (patients number 2, 3, 4, 5, 6, 7, 8). Two patients received 100 million units (patients number 11 and 12) and the single dose was 5 million units (20 injections). One patient (no. 10) received 120 million units in 20 injections. One patient (no. 9) received 24 million units in 12 injecitons, and he received an additional 10 million units in 2 intralymphatic applications. After surgery one patient (no. 13) received only one intralymphatic injection of 5 million units. Patients nos. 1, 7, 9, 13 received interferon post-surgically.

Clinical evaluation of results

Clinical results were evaluated on the basis of the tumour size before and after therapy with interferon.

Pathohistological evaluation

The pathohistological confirmation of the clinical diagnosis was done on the bioptic material. Excised tumour represented the bioptic material.

Immunohistological evaluation

The histological and histochemical evaluation of results took into consideration primarily lymphoid cells and macrophages in stroma:

0 = no response, nonreactive stroma
\+ = poor response, scattered lymphoid cells
++ = moderate response, numerous lymphoid cells, narrow infiltration areas, few macrophages
+++ = good response, massive infiltration of lymphoid cells, numerous macrophages
++++ = very good response, massive infiltration and formation of lymphoid nodules in stroma, numerous macrophages.

RESULTS

Therapy results in melanoma patients with recombinant interferon are shown in the Table 2. According to clinical evaluation of tumour size, it is observed that in each individual patient after interferon therapy, the size of the tumour is reduced in way that the longer and shorter diameters of the tumour are decreased.

Not only were both diameters reduced after therapy with interferon, but also the appearance of the tumour changed. Tumours were shrunk, length and width were reduced as well as the height of the tumour, i.e. all three diameters, the surface peeled off and eges were becoming clearly bordered. In patient no. 6, a melanoma of large dimensions (50 = 55 mm.) was so strongly bordered from the healthy tissue after application of interferon that the surgeon peeled it off from the planta of the right foot and amputation of the right foot was thus avoided owing to the interferon treatment.

The immunohistological grade of melanomas with metastases after therapy with interferon showed a poor response (+) after four weeks of treatment in patients no. 3, 5, 8 and 11. A moderate response (++) was shown after therapy with interferon in patients no. 4 and 12 (four weeks of treatment). A good response (+++) after therapy with interferon was shown in patients no. 6 and 10 (four weeks of treatment). A very good response (++++) after therapy with interferon was shown in patients no. 1, 9 and 13. These three patients received interferon post-surgically into the scar.

Patients no. 4, 5 and 8 died. Patient no. 4 received interferon in November 1985 and died in June, 1986 with two metastatic tumours of the liver and lungs. The immunohistological response was moderate (++). Patient no. 5 received interferon in December 1985 and died in February, 1987. In this patient (no. 5), interferon was syringed into the second metastatic tumour. Before that, he was operated on for his first primary tumour, and after 6 months surgery was done on the first primary tumour. The immunohistological response was poor (+). Patient no. 8 received interferon in December, 1985, and died in June, 1986. The immunohistological response was poor (+). In this patient (no. 8) interferon was syringed into the metastatic tumour of the right upper leg. Before that, surgery was done on 2 metastatic tumours of the right axilla and abdomen.

As a rule, following the applications of 5 million units of interferon, and some the next day, all patients develop higher body temperatures up to 38 °C. Besides temperature shivers occurs. This settles after an hour or two without intervention. With the intervention of antipyretics, the settlement comes much earlier.

Locally, light erythema is evident with the regional irritation in context of tolerable itching.

DISCUSSION

In Robinson, W. A., et al. (1986), in a clinical trial, 4 of 40 patients (10%) with cutaneous primaries achieved complete remission, and 6 further melanoma patients (15%) had partial remissions for a combined response rate of 25%. Two patients remain in complete remission 15 and 32 months after starting treatment. Interferon was given in a dosage of 10 million IU/M^2 subcutaneously three times per week for one year. Response rates of 10 and 20 per cent have been reported by most investigators using different interferons and different dosing schedules (Krown, S. E., et al. 1984; Retsas, S. T., et al. 1983; Kirkwood, J. M., et al. 1985). A survey of the literature shows a low but significant response rate in patients with disseminated melanomas using recombinant alpha interferons (Carter, 1984; Kleeberg, 1986; Kirkwood, 1986; Legha, 1986; Spiegel, 1987; Creagan, 1987). The overall experience suggests that alpha interferon has some activity against melanoma with a response rate between 12% and 20%.

A small proportion of patients with disseminated malignant melanomas may achieve a complete response and some of these may have a prolonged remission (5 of 50 patients — Kerr, R., et al. 1985; 4 of 96 patients — Creagen, E. T.,et al. 1986). Some of these patients have already exceeded the median

of 9 months. The objective response rate seen in some of these studies rivals that of the best available chemotherapy for melanoma (Luikart, S. D., et al. 1984; Hill, G. I., et al. 1984).

The interferon antitumour activity against melanoma mentioned above had been achieved by intramuscular or intravenous administration. In our clinical trials interferon was administered locally and intralesionally. Local administration of interferon requires smaller concentrations of interferon and reactions are smaller (Ikić, D., 1975 and Ikić, D., et al. 1975).

Side reactions are an influenza — like syndrome which develops shortly after the beginning of the therapy and which is dose dependent. Symptoms include fever, chills, muscle aches and headaches. In our cases symptoms settle after an hour or two without intervention and become less noticeable with continued use of interferon. Interferon is likely to be of only limited value for the treatment of patients with advenced melanoma and a high tumour burden. One assumes a higher effectiveness early in the course of illness. One of our patients (no. 6) avoided the amputation of the right leg owing to interferon treatment.

All the aforementioned results of interferon administration justify further research of interferon in the treatment of melanoma.

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SUMMARY

Therapy results in melanoma patients with recombinant interferon are shown in the Table 2. According to the clinical evaluation of tumour size, it is observed that in each patient after interferon therapy, the size of the tumour is reduced in way that the longer and shorter diameters of the tumour are decreased.

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As a rule, following application of 5 million units of interferon, and some the next day all patients develop higher body temperatures up to 38 °C. In addition to high temperatures, shivering also occurs. This settles after an hour or two without intervention. With the intervention of antipyretics, the settlement comes on much earlier.

SAŽETAK

MELANOMI. TERAPIJA SA REKOMBINANTNIM INTERFERONOM HuIFN ALPHA 2c. PRETHODNO SAOPĆENJE

Rezultati terapije melanoma rekombinantnim interferonom prikazani su u tabeli 1 i 2. Prema kliničkoj evaluaciji veličine tumora vidimo da je kod svakog pacijenta nakon terapije interferonom veličina tumora smanjena tako da su i duži i kraći dijametar tumora umanjeni.

Ne samo da su oba dijametra poslije terapije interferonom umanjena nego je promijenjen i izgled tumora. Tumori su smežurani (ustvari umanjena je ne samo dužina i širina tumora nego i njegova visina, tj. sva tri dijametra), površina se ljušti, a rubovi postaju oštro ograničeni. Kod pacijenta br. 6 melanom velikih dimenzija bio je nakon primjene interferona umanjen, oštro ograničen od zdravog tkiva, pa ga je kirurg odvojio od tabana desne noge. Prije primjene interferona pacijentu je trebala biti amputirana desna noga iznad potkoljenice. Nakon primjene interferona noga je sačuvana. Pacijent normalno obavlja svoje težačke poslove, duže od 3 godine. U pravilu kod svih pacijenata nakon primjene doze od 5 miliona jedinica, a kod nekih drugi dan dolazi do povišenja temperature do 38 °C. Uz temperaturu javlja se i tresavica. Temperatura se ubrzo smiruje bez intervencije.

Table 1

MELANOMAS

Patient No.	Sex	Year of birth	Date starting therapy	Tumour site	PH findings after HLI
1***	F	1951	16. 09. 1985	Left axilla	Granulationes
2***	F	1951	04. 11. 1985	Right upper arm	Granulationes
3	F	1912	09. 10. 1985	Left submandibulary	Melanoma metastaticum
4**	F	1948	07. 11. 1985	Right lower leg	Melanoma metastaticum
5**	M	1927	16. 12. 1985	Right retromandibulary	Melanoma metastaticum
6	M	1907	20. 11 1985	Planta of right leg	Melanoma
7***	M	1931	01. 11. 1985	Neck	Keratosis seborrhoica
8**	F	1944	16. 12. 1985	Right upper leg	Melanoma metastaticum
9***	M	1956	06. 01. 1986	Left upper leg	Granulationes
10	F	1942	27. 02. 1986	Right lower arm	Melanoma
11	F	1941	07. 04. 1986	Left lower leg	Melanoma nodulare
12	F	1939	18. 11. 1986	Back	Melanoma
13***	F	1938	09. 12. 1985	Anocutaneous border	Granulationes

Table 2

MELANOMAS

Patient No.	Duration of treat.	No. of applic.	Single dose ($\times 10^6$)	Total units ($\times 10^6$)	Clinical**** grade	Size of TM (cm) before treatment	Size of TM (cm) after treatment	Immuno histol. grade
1	2 weeks	10***	2	20	$T_1N_0M_0$			++++
2	4 weeks	20***	2	40	$T_2N_0M_0$			+++
3	4 weeks	20	2	40	$T_3N_1M_0$	3.4 × 5.6	2.7 × 4.5	+
4**	4 weeks	20	2	40	$T_3N_0M_0$	2.2 × 3.1	1.5 × 2.0	++
5**	4 weeks	20	2	40	$T_3N_1M_0$	3.2 × 4.0	2.8 × 3.4	+
6	4 weeks	20	2	40	$T_3N_0M_0$	5.0 × 5.5	3.0 × 3.5	+++
7	4 weeks	20***	2	40	$T_3N_0M_0$			+++
8**	4 weeks	20	2	40	$T_3N_1M_0$	3.5 × 5.1	2.7 × 4.5	+
9	12 days	12* 2***	2 5	34	$T_3N_1M_0$			++++
10	4 weeks	20	6	120	$T_1N_0M_0$	1.0 × 1.0	0.7 × 0.7	+++
11	4 weeks	20	5	100	$T_1N_0M_0$	1.2 × 1.2	1.0 × 1.0	+
12	4 weeks	20	5	100	$T_1N_0M_0$	0.8 × 0.8	0.6 × 0.6	++
13	1 day	1* ***	5	5				++++

* Patients No. 9 and No. 13 received also interferon intralymphatically. No. 9 received 10 million units. No. 13 received 5 million units.
** Patient No. 4 died in June, 1986., No. 5 died in February, 1987., No. 8 died in June, 1986.
*** No. 1, 2, 7, 9, 13 melanoma patients received interferon postsurgically.
**** On basis of anamnesis.

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